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ANTHRACNOSE, ALTERNARIOSE AND BOTRYTIS ROT OF THE SNOWBERRY

PART I

ANTHRACNOSE OF THE SNOWBERRY CAUSED BY GLOMERELLA RUFOMACULANS

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(WITH PLATES 15–19 AND 5 TEXT FIGURES)

During the autumns of 1926, 1927, and 1928, fruits on the snowberry shrub (*Symphoricarpos albus* L.) Blake var. *laevigatus* (Fernald) Blake turned a brown color and dropped prematurely. At first, it was thought that this discoloration was due to a freezing of the fruits (snowberries) before they had matured. However, in the autumn of 1928, a careful diagnosis revealed sporulating acervuli of a *Gloeosporium* parasitizing several diseased snowberries (PLATE 15, *A* and *B*).

Stewart (1910) reported a *Gloeosporium* which parasitized snowberries but he did not assign a species to the organism. Barrus and Horsfall (1928) stated that they isolated a *Gloeosporium* from the snowberry but were unable to obtain infection when they sprayed the fruits with conidia. From this brief review of the available literature, it appeared an investigation should be undertaken to solve the following problems:

1. How prevalent and widely disseminated is this disease?
2. Is the disease caused by a *Gloeosporium?*

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3. If so, what Latin binomial should be assigned to the fungus in its perfect and imperfect stages?

4. What is the life history of the organism?

5. What fruits other than the snowberry, if any, are parasitized?

6. From a study of the life history of the fungus and development of the disease, what controls could be practiced?

SYMPTOMS AND SEASONAL OCCURRENCE OF THE CONIDIAL STAGE

In 1928, several fruits infected with anthracnose were first observed during the month of September but during the latter part of October an average of sixty per cent of the fruits on all shrubs examined were infected. The initial symptoms appeared as minute hazel-colored areas which continued to enlarge until the whole snowberry was a cinnamon-rufous color. Later, these infected snowberries turned shades of hazel but when incubated for 10 days under favorable conditions, they were dotted with small, flesh-colored flecks which were conidial masses or acervuli rupturing the epidermis. Finally, these acervuli turned an olive black or dead black, which was due to an outgrowth and change in color of the mycelium (PLATE 15, FIGS. *A* AND *B*, PLATE 16, FIG. *A*, PLATE 19, FIG. *B*, 7, 8, 9). Most of the snowberries infected with the *Gloeosporium* shriveled and dropped to the ground or were easily beaten from the shrubs by wind, rain and snow. After detached snowberries had remained on the ground some time, most of them turned black and mummified (PLATE 18, FIG. *B*, PLATE 19, FIG. *B*, 9).

However, some of the fruits were parasitized by *Penicillium* sp., *Alternaria* sp., and *Botrytis* sp., and then the snowberries were of a steel gray, a chamois or a yellowish tinge, which was often accompanied by a wet rot (PLATE 18, FIG. *A*, 3, 4, 7, 8, and PLATE 19, FIG. *B*).

In April 1929, thirty-six infected snowberries which had been buried under leaves at the base of shrubs and overwintered there during 1928–1929 were brought to the laboratory and placed in a moist chamber. Numerous acervuli bearing viable conidia were observed on eight of them. Furthermore, three weeks later, acervuli bearing viable conidia were also observed on snowberries

which had not been placed in a moist chamber but had remained in their natural environment; on shrubs, in the open.

The flesh-colored spore masses or acervuli often appeared near the veins and on folded surfaces of leaves not exposed to the open air. Excised leaves and fruits which had been inoculated and afterwards incubated in a moist chamber showed symptoms of the disease within eight to eighteen days. These infected areas on the leaf bore a dark or black color as if stained by a dye.

The bark of infected twigs possessed a water-soaked, dark appearance which was ruptured in various places by sporulating acervuli. Here the spore masses appeared flesh-colored as on fruits and leaves. On the shrubs under observation, the tips of these infected twigs did not remain alive during the following season, but generally died back about two or three nodes from the infected inflorescence. These infected tips of twigs did not produce viable conidia of a *Gloeosporium* during the following season when they were dead (TEXT FIGURE 2, *A*, 1; *B*, 0; *C*, 1).

Cankers seldom formed on the stems of the plants under observation. In 1928, no cankers could be located, but in 1929 several cankers were observed on the current season's wood. These occurred near the middle of the internodes, causing a swelling and enlargement in diameter (1.5 to 2 times), presenting the appearance of a healing wound. The wood of the canker was darker in color, cracked lengthwise of the stem and, in October, bore sporulating acervuli with viable conidia around the margins of the rift. In one case there were fruiting bodies of the same size as those of *Glomerella* perithecia but no asci could be found within them as they had reached maturity and were disintegrating.

These observations showed that symptoms of the disease were not in evidence during the blossoming period of July 1928 and 1929 but first appeared on the snowberries in the autumn or about September first. The fungus overwintered in snowberries which had been buried under organic materials and sporulated during the following spring, producing viable conidia; the ascogenous stage was also located in diseased snowberries which had remained outdoors under natural conditions. Furthermore, the first snowberries infected, those at the base of the fruit cluster,

sometimes bore perithecia while on the shrub. Specimens were placed in the Cryptogamic Herbarium of the Massachusetts Agricultural College under the accession number 3155 (PLATE 18, *C*).

GEOGRAPHICAL DISTRIBUTION AND ECONOMIC IMPORTANCE

In 1928, diseased snowberries were observed on each snowberry shrub examined. The disease was widely distributed in Massachusetts, Connecticut, New Hampshire, Vermont, and New York State. Pathologists reported its presence in Iowa, Illinois, Wisconsin, Michigan, Ohio, New Jersey and Pennsylvania.[1] So it would seem that in 1928 the disease was prevalent in northeastern United States. In 1929 the disease reoccurred and was reported prevalent in Vermont, Massachusetts, Connecticut, and New Hampshire.

The snowberry shrub is extensively used in landscaping around dwellings, in gardens, private estates and parks. Its principal beauty lies in the abundance of beautiful, snowy-white fruits from which it derived its common name, snowberry. The shrub is symmetrical, ornamental with and without foliage, is easily cultivated and highly prized by landscape gardeners and others for plantings on estates and for ornamentation in general. The fungus robs the plants of a greater portion of their beauty, as it discolors the leaves and snowberries, causing them to drop prematurely; infects and kills some of the young wood, thereby rendering the plants unhealthy as well as unsightly. Furthermore, the fungus killed seeds which could have been used for propagative purposes. If the fungus should continue to render the fruits as unsightly as it did in 1927, 1928 and 1929, the shrub would probably lose more than half of its economic value as an ornamental in northeastern United States.

ISOLATION, CULTURING AND INOCULATION WITH THE PATHOGEN

The fungus was isolated by four methods:

1. Transfer of mycelium. Diseased snowberries were incubated in moist chambers until mycelium of the fungus appeared

[1] Pathologists stated in personal conversation that they believed they had seen the disease on snowberries cultivated in several of the states mentioned.

on their surfaces; then portions of this mycelium were transferred to tubes of potato-dextrose agar.

2. Monosporous culturing. Employing bacteriological methods, spore dilutions were prepared and poured on agar. When a single conidium was observed germinating in a clear microscopic field, it was transferred, on a block of agar, to a tube of potato-dextrose agar.

3. Spore streaking. A sterile needle was dipped in an abundantly sporulating acervulus and the conidia transferred, to tubes of slanted, potato-dextrose agar, by drawing the needle along its surface.

4. Tissue culturing. The surfaces of diseased snowberries and leaves were sterilized, small portions of diseased tissue together with some of the adjoining healthy tissue were removed under aseptic conditions and transferred to tubes of slanted potato-dextrose agar.

By each method, pure cultures of the fungus were readily procured. After incubating a transfer culture for 5 to 10 days at 20° C., the mycelium, which at first was a light-gray, turned to a shade of brown and at the center of the culture showed a black area. Often, the cultures had a tinge of olive-green when observed from the under side. The culture also bore the characteristic flesh-colored spore masses with conidia on acervuli.

To establish the pathogenicity, physiology and host range of the fungus, various fruits were inoculated by spraying their punctured and unpunctured, sterile surfaces with conidia from pure cultures and from sporulating snowberries. Mycelium was also laid on sterile surfaces and inserted under the epidermis of the fruits. Then the inoculated fruits were incubated in Coplin jars lined with dampened filter paper and stored at 20° C. A list of the fruits inoculated follows:

1. Snowberry
2. Quince
3. Pear
4. Apple
   - Baldwin
   - McIntosh
6. Tomato
7. Banana
8. Cranberry
9. Peppers
   - Green
   - Red

5. Grapes
   Niagara
   Tokay
10. Indian currant (*Symphoricarpos orbiculatus* Moench)

Leaves and stems of the snowberry were also inoculated and checks prepared for each inoculation made.

Infection was observed in each fruit inoculated except the cranberry, which together with the checks remained healthy. Furthermore, sporulation occurred on each of the surfaces but most abundantly on the quince, apple and banana. The development of the infection areas on the green pepper was very slow, sporulation not being observed until the third week following inoculation, while spores were observed on the snowberries 6 days and on other fruits 5 to 12 days after inoculation. When conidia were spread on the snowberry, apple, pear, and banana, three fourths of the inoculated areas showed infection; on the other susceptible hosts, about one third of the inoculations showed infection. Infection was also obtained when mycelium was placed in punctures of the susceptible hosts.

Excised leaves were inoculated by Clinton's method (**2**) but were very slow to show sporulation, which occurred either the second or third week following the inoculation. On leaves, the fungus behaved more like a facultative saprophyte than a true parasite. It seemed to sporulate best when the leaves were partly decayed.

At the present time, it is too early to predict the results of the twig inoculations.

PATHOLOGICAL ANATOMY

Stained sections on prepared slides showed that the surface of the snowberry is covered by a cuticularized layer varying from three to eight microns in thickness. Under this layer of cutin is the epidermis, which consists of a layer of polyhedral cells with comparatively thick walls which average six microns in thickness (TEXT FIGURE 1, 16, and PLATE 15, FIGS. *C*, *D*, *E*). The walls of the epidermal cells are suberized and this manifests itself as small biscuit-shaped masses of suberin about as thick as the cell wall itself (PLATE 15, FIG. *F*). In old snowberries, these suberin

thickenings were fastened to each inner wall of the epidermal cells. As seen through the microscope, this suberized material gave the cell wall a rough appearance and probably has much to

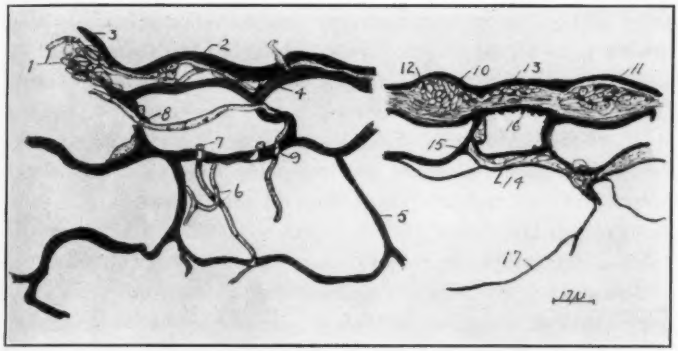

FIG. 1. Drawings of the outer cells in a diseased snowberry showing the position of the hyphae, beginning of an acervulus and penetration of the cell walls by hyphae of *Gloeosporium rufomaculans* collected in November. (Traced by aid of a camera lucida from triple stained sections permanently mounted. numbers 1 to 9 are from one specimen and 10 to 17 from another). No. 1, 2, 11; Hyphae between cutin and epidermal cell walls; No. 3, 10. Cutinous layer. No. 4, 15. Walls of epidermal cells. The pits are omitted; No. 5, 17. Walls of mesocarpal cells; No. 6. Infracellular mycelium of the mesocarp; No. 7; Haustorium; No. 8. Hypha entering through a pit; No. 9. Hypha penetrating the cell wall where no pit could be detected; No. 12. Early formation of an acervulus; No. 13. Deliquescing hyphae; No. 14. Hyphae between epidermal and mesocarpal cells; No. 16. Suberized membrane of an epidermal cell.

do with the snow-white appearance of the snowberry as the material itself is a clear, white color. It was insoluble in concentrated sulfuric acid but soluble in an aqueous solution of potassium hydroxide acting for 4 to 12 hours. It did not turn blue but brown when submerged in a solution of potassium iodide. The suberin was stained green by a hot alcoholic solution of chlorophyll [2] acting for one hour, while the cellulose of the cell walls remained hyaline. The suberin material was drawn from

[2] The chlorophyll solution was prepared by adding, to 300 cc. of 95 per cent alcohol, 75 grams of tomato leaves removed at noon on a clear day. The preparation was boiled gently for 15 minutes, 225 cc. of the solution was decanted and thin sections of epidermal cells of the snowberry were submerged in this solution and placed in a dark chamber for one hour.

the cell wall toward the center of the cell by plasmolysis with glycerin, which shows it is a suberized protoplasmic membrane appearing as a part of the cell wall. Furthermore, this suberin appeared to be laid down in these cells about the time the snowberry changes from its green color, due to chloroplastids in the mesocarp, to the white waxy color. A layer of suberin appeared to be deposited within the upper walls of the epidermal cells (TEXT FIGURE 1, 16).

Under the epidermal layer is the mesocarp, which is composed, for the most part, of many layers of very large cells with thin, smooth, hyaline walls averaging three microns in thickness (TEXT FIGURE 1, 5, 17).

Scattered within the mesocarp are the fibro-vascular bundles and near the center are two seeds in large chambers.

The hyphae and germ tubes from conidia of the fungus penetrated the cuticle or passed unobstructed through stomata into the snowberry (PLATE 15, FIG. *D*) and multiplied between cuticle and epidermal cells, forming mats of sclerotia which later produced conidiophores and conidia which comprised the acervuli. The rufous color manifested in the external symptoms of the disease is believed to be due to the color of these maturing hyphae as seen through the cuticle. However, when the mycelium turns black, the old infected snowberries also appear black (PLATE 16, FIG. *A*, 6, 12, 19). Later, the hyphae pass from cell to cell by dissolving the suberin or passing through pits in the epidermal cell walls. Finally the hyphae reach the mesocarp and the central chamber containing the seeds (TEXT FIGURE 1, NUMBERS 11, 12; PLATE 19, *A*).

The initial infection of snowberries in the autumn would seem to be produced by hyphae coming from infected stems and flower stalks through pedicels into the berries and thus arriving at the mesocarp before reaching epidermal cells.

Observations in 1928 showed that the disease first appeared in the autumn, September, and that in most cases the oldest snowberries or the ones nearest to the main stems were first to show symptoms of anthracnose. Furthermore, twigs bearing diseased fruits died back from the tips a distance of two to three nodes. These observations seemed to furnish evidence of systemic,

perennial mycelium. An attempt was made to detect the fungus within the twigs and trace it to its destination. In July and August, 1929, young twigs were hand-sectioned and the sections mounted in green lactophenol. These twigs had grown from 1928 twigs which had borne diseased snowberries.

In July, the pith cells in the tips of the young twigs were yet filled with starch grains while those next to the old parent stem were somewhat disintegrated, as the starch had been removed. Here, hyphae of a fungus were present in abundance. A *Gloeosporium* was cultured from sections of those stems which had been externally sterilized and incubated on potato-dextrose agar. Later, hyphae could be detected in the medullary rays of the wood.

To determine the presence of this fungus within stems, the exterior of four stems bearing diseased snowberries was sterilized by submerging them for 10 minutes in an aqueous solution of bichloride of mercury 1 : 1000 with the addition of 2 cc. of HCl and then rinsed in sterile distilled water. Sections were removed under aseptic conditions and incubated on potato-dextrose agar (TEXT FIGURE 2).

The results may be summarized:

1. The dead wood at the tips did not contain a viable *Gloeosporium* but near the lower node (TEXT FIGURE 2, *A*, NUMBERS 1, 2) two sections showed the presence of a viable *Gloeosporium*.

2. Most of the internodes contained hyphae of a *Gloeosporium* (*A* and *B*, 7, 10, 11, 12, 13, 20, 24, 25, 26); the cultures from *C* (6, 11, 12, 13) did not sporulate well save 11, but possessed the characteristic mycelium. However, the cultures contained more actively growing mycelium and sporulated best from the nodes and from older wood.

3. Most of the twigs of the current year's growth contained hyphae of the *Gloeosporium* from base to tip. An exception was that shown in Text figure 2, *B*, 41 to 45. Here, the mycelium did not sporulate except in 44, which showed a *Gloeosporium*. In twig *C*, sporulation around sections incubated from the lower twigs was poor or none at all (NUMBERS 9, 10, 14, 15).

4. Eighty per cent of the autumn buds contained hyphae of a *Gloeosporium*. Buds were removed from branches containing

infected berries (*D*, 5, 6, 8) and one from each of eight nodes on a branch bearing apparently healthy berries (*D*, 7). Buds from each of these twigs contained *Gloeosporium* mycelium most of which sporulated when cultured.

FIG. 2. Diagrams showing where sections were removed from sterilized snowberry twigs. These sections were incubated on agar to detect *Glomerella rufomaculans*, if present, within the tissues during September and October, 1929. Numbered triangles denote the parts cultured; diseased fruits and ovaries are shown in black. A. A branch with dead twigs of 1928 (1, 2) and live twigs bearing diseased snowberries (5, 6, 9); B. Similar to A, only collected two weeks later. The twigs on this branch bore diseased fruit, save 46 and 47. Sections 21 to 23 were removed to determine whether mycelium of *Gloeosporium rufomaculans* could be present in a twig and not apparently infect the fruit; C. Similar to B, a check; D. Buds were removed from these twigs (5, 6, 8) bearing diseased berries; also, from 7, which bore apparently healthy berries. Culturing methods showed a *Gloeosporium* present in most of them.

5. Flower stalks (floral axles) and pedicels from the tips of three twigs with diseased snowberries were externally sterilized and incubated on agar. A *Gloeosporium* was evident in each culture.

6. Hand sections showed hyphae within the outer bark of twigs and between the outer bud scales within the buds.

From the above observations and data, it would seem that

initial infections generally occur in bud scales or the bark. From these parts, the hyphae penetrated the pith cells of the new growth or occupied the hollow cavity of the maturing stem from which they entered the flower stalk and pedicel into the berry. Conditions are not suitable for the passing of hyphae from the stem into the fruit earlier in the season than September. Furthermore, hyphae can occupy an area around the lenticels. It was not decided whether hyphae from the pith could penetrate the medullary rays and enter the bark, but the writer believes they can, as hyphae at one node seemed to do so. This being true, the fungus might advance from old growth into new.

In the twigs, the acervuli were mostly confined to the current year's growth. Diseased twigs bearing acervuli were collected in December and free-hand sections made and mounted in lactophenol containing acid green. By this method, the hyphae were well differentiated from the wood and bark elements, for they were stained a brilliant green. In these sections, the hyphae were most evident just beneath the epidermis; sometimes occupying areas from the outer corky bark cells to the cambium. Many of the cells were hypertrophied and filled with the sclerotial masses which had ruptured the outer layers of bark and formed sporulating acervuli. These acervuli appeared black at the ends but in sections of acervuli sporulating on twigs which had not been removed from moist chambers the spore masses were sometimes a flesh-color which is characteristic of this *Gloeosporium*.

Acervuli ruptured the epidermis, appeared black, were scattered but not numerous. The tips of infected twigs soon died, together with their winter buds. However, buds on apparently healthy wood directly below the dead tips of twigs formed new growth.

Permanent, stained mounts were made from microtome sections of infected leaves of the snowberry plants. In the sections examined, the hyphae penetrated the palisade and mesophyll cells but were most numerous around epidermal cells. The hyphae formed sclerotial masses which were the sources of acervuli (TEXT FIGURE 3, NUMBERS 7, 13, 14). In the leaves examined,every tissue except the xylem was penetrated by hyphae of the *Gloeosporium*.

CONIDIAL STAGE OF THE PATHOGEN

Free-hand sections of dried, diseased snowberries were mounted in green lactophenol and 20 cells of the parasitic mycelium

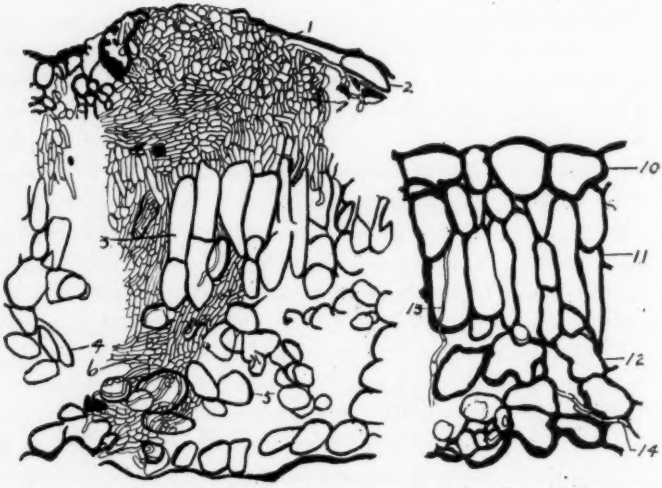

FIG. 3. Outline drawings showing cross-sections of a diseased snowberry leaf collected in October. (Tracings by aid of a camera lucida, from triple stained sections permanently mounted; numbers 1 to 6 were near the midrib and 10 to 14, two millimeters nearer the margin of the same leaf). No. 1. Cutin on the upper surface; No. 2, 10. Epidermal cell; No. 3, 11. Palisade cell; No. 4, 5, 12. Mesophyll cell; No. 6, 14. Hyphae in the air spaces between mesophyll cells; No. 7. Beginning of an acervulus, the hyphae have raised the cutin and epidermal layers; No. 13. Hyphae penetrating a palisade cell.

measured; the lengths varied from 17 to 30 microns; diameter, 1.5 to 2.5 microns. Sometimes several hyphae coalesced and under certain conditions they deliquesced. Colors of hyphae in culture: at first, light-gray, finally a dark olive-black to black. Very often a reddish hue appeared in rapid growing cultures. No clamp connections were observed.

The acervuli averaged 101 microns in width; 58 microns deep; often several coalesced. The conidiophores were constricted at the apex where conidia were abstricted; they averaged 2×20 microns (PLATE 16, FIGS. *B*, *C*). Masses of fresh conidia were pinkish (varying from a flesh-color to a hazel) but individual

conidia were mostly hyaline when observed with a microscope or sometimes bore a wash of brown when old; 100 fresh conidia were collected from several different hosts two weeks after inoculation and the conidia measured (PLATE 16, FIG. *D*).

A tabulation of standards and limits of variation in the sizes of conidia for *Gloeosporium rufomaculans* obtained from different cultures and hosts. Sizes reported by other investigators are also listed for comparisons.

Sources of Conidia	Standard in Microns	Limits of Variation in Microns
Agar cultures (Snowberry)		
From conidia	5.4 × 15	4.5–7.2 × 12.6–20[3]
From ascospores	5.4 × 20	5.4–6.3 × 12.6–28.8
Banana	5 × 19.4	
Apple	5 × 15.4	
Tomato	5.7 × 15.4	
Quince	5.8 × 15.0	
Grapes—Tokay, Niagara	5.7 × 15.5	
Stewart's *Gloeosporium* sp.	4 × 6–8	
Saccardo (*G. fructigenum* Berk.)	5–6 × 20–30	
Spaulding, and von Schrenk (*G. rufomaculans*)	4–5 × 12–16	3.5–7 × 10–28
Craig (*G. fructigenum*)		5–7 × 10–20

From the above list of measurements, it is to be noted that conidia from a culture transferred from germinating ascospores were longer than those from a monosporous culture from a conidium. Furthermore, conidia on bananas were noticeably longer than those from the other hosts. The conidia from the snowberry seemed to be wider and much longer than those reported by Stewart but compare favorably with those from the apple reported by Spaulding and von Schrenk.

From the preceding description of the pathogen it is to be noted that the fungus is a *Gloeosporium*, as the "spores ooze out in pink masses" (Stoneman), and the acervuli, conidiophores and conidia are characteristic of the genus and the mycelium possesses the culturing characteristics of *Gloeosporium rufomaculans* (Berk.) Thüm. This is a form-species which parasitizes similar hosts, as shown by the inoculations performed, and the morphology described is within reasonable limits, for the fungus parasitizing the snowberry.

[3] When conidia from snowberries were dried for 3 weeks, then mounted in water, they measured 2.8–6 × 9–18.5 μ. Standard (5 × 12.6 μ).

THE ASCOGENOUS STAGE

The surfaces of snowberries were sterilized, inoculated with conidia from a pure culture, stored in sterilized Coplin jars containing wet filter paper and incubated under aseptic conditions at 20° C. Also, agar plate cultures and snowberries inoculated as described elsewhere in this article were incubated at the same time and under the same conditions.

After these inoculated snowberries had incubated for 4 to 6 weeks, perithecia formed on their surfaces. Perithecia also formed in the agar plate cultures after they had been incubated 8 weeks. Perithecia in the agar plate cultures were all sterile or abortive save two which contained immature asci without ascospores. The walls of these perithecia were exceptionally brittle, carbonaceous and lacked much of the floccose layers associated with perithecia in nature. These agar cultures were stored in full sunlight and slowly dried, but no decided change in the formation of perithecia was noted.

In one culture where the snowberry was inoculated, perithecia formed on the fifth week of incubation. These perithecia were on a floccose stroma but not buried in it. The stroma averaged 60 microns deep and was located on the surface of the snowberry (PLATE 17, FIGS. *A*, *B*, *C*). Perithecia were most numerous within wrinkles or between folds or those surfaces which did not have free access to the air. Perithecia bearing viable ascospores were collected in snowberries on diseased shrubs in 1928 and in 1929, thus showing that the ascogenous stage may appear late in the autumn while the snowberries are attached to the shrubs. Perithecia were also found on old mummies which had overwintered under leaves. Viable ascospores were expelled in April.

Hand sections of matured perithecia were mounted in green lactophenol (PLATE 17, FIG. *D*). The following table shows the average measurements in microns of 25 perithecia and asci, and 100 ascospores, together with measurements reported by two other investigators:

The perithecia generally occurred singly but sometimes two or more were joined at their bases. The wall was carbonaceous and

TABLE

Authority	Substrate	Width of Base	Total Height	Beak		Asci	Ascospores
				Width	Length		
The writer	Snowberry	164–312 [4]	246–451	50–70	50–110	10–14.4 × 63–72	5.4–7.2 × 14.4–20
		213 [5]	344	57	81	12 × 72	6 × 16
Spaulding and von Schrenk	Apple agar	—	125–250	—	—	9 × 55–70	3.5–5 × 12–22
Craig	—	—	—	—	—	16–64	5–7 × 20–28

[4] Limits of variation.
[5] Standards based on 60 per cent or more of the individuals examined.

somewhat brittle. From the table above it is to be noted that they averaged 213 microns wide at the base and 344 microns in height. They had three layers: an exterior consisting of floccose hyphae, a central of thick-walled hyphae, and an interior of hyaline thin-walled hyphae. Most of the perithecia on snowberries possessed distinct beaks which averaged 57 microns wide at the base and 81 microns long or occupied about one fourth of the total height of the perithecium. A few hyphae, "hairs," were sometimes found on the beak. The shapes of perithecia and beaks differed; a globose perithecium with a conical beak; a cylindrical perithecium with a conical and reflexed beak; both perithecium and beak forming an ovoid, and indistinctly defined from each other (PLATE 17, FIGS. *A*, *B*, *C;* PLATE 18, FIGS. *B*, *C*).

The asci were oblong, clavate to subclavate, blue at the tip, opened by a pore through the thick-walled tip which may possess a lid. They were mostly attached to the base of the perithecium and sometimes pedicellate (PLATE 18, FIGS. *D*, *E*, *F*). In some perithecia a hyaline material was located between asci and in this material were very minute fibers suggesting paraphyses. However, periphyses press against the pore of the perithecium and aid the escape of its contents. The ascus wall did not stain with potassium iodide while the contents of the ascus was colored an amber.

The ascospores in the ascus were alantoid, hyaline, uniseriate, non-septate. However, they germinated immediately, even in the ascus, and then some were septate. The asci averaged 12 microns wide and 72 microns long, while the ascospores averaged 6 microns wide and 16 microns long. The ascospores are sometimes forced into the tip of the ascus from which some escaped through a pore (PLATE 18, FIGS. *D*, *E*, *F*). The ascospores are short lived; very much like the whole perithecium in this respect. In cultures, asci and ascospores disappeared in 9 days so that the culture was useless for experimentation. Conidia and ascospores were much alike in appearance and were sometimes found in the same culture. However, the ascospores were allantoid (sausage-shaped) while conidia were cylindrical without the crescent bend; ascospores appeared narrower and generally bore a blue tinge while the conidia were hyaline. On account of

these two spore forms of the fungus being so much alike and so closely associated it was considered unsafe to make a monosporous culture from single ascospores; so asci were isolated and when the germ tubes of the ascospores had started to penetrate the ascus wall, the ascus with germinating ascospores was transferred to a culture tube of potato-dextrose agar. Ten such isolations were made and nine produced the characteristic conidial stage. Five snowberries were inoculated with asci containing germinating ascospores and three showed the characteristic disease together with the conidia of a *Gloeosporium*. Thus the life cycle of the polymorphous fungus was established from conidial to the ascogenous or perfect stage from which it returned to the original conidial or propagative stage.

According to the evidence collected regarding the physiology and morphology of the fungus, it is *Glomerella rufomaculans* (Berk.) Spauld. & Schrenk, or in its conidial form *Gloeosporium rufomaculans* (Berk.) Thüm. (*Gloeosporium fructigenum* Berk.).

DISCUSSION

Most of the perithecia cultured on the snowberries had beaks which would seem to be a constant morphological character of the organism on this host. However, Spaulding and von Schrenk (p. 24) stated that the perithecia of *G. rufomaculans* have no beaks. They obtained their perithecia from cultures on apple agar which had been stored at 33° to 38° C. The writer obtained perithecia on snowberries which had been inoculated, incubated at 20° to 23° C., and exposed to natural conditions of light and air. Furthermore, perithecia were collected on infected snowberries in the open where they developed entirely under natural environment. These perithecia which developed under natural conditions could not be distinguished morphologically from those which developed under controlled laboratory conditions.

The fact that perithecia on snowberries were beaked, the walls not entirely carbonaceous but of three layers, of 344 microns in height instead of 250 microns as in the type, with asci and ascospores slightly larger, is no more than to be expected when one considers the afore-mentioned variation of conditions under which the two forms were cultured. However, these morphological

variations are not sufficient to establish a new species since the physiology of the two forms appears the same. So *Glomerella rufomaculans* seems to be the proper Latin binomial for the fungus causing anthracnose of the snowberry.

CONTROLS

Two groups of shrubs, each situated on the south side of a building but well separated by a large entrance, were employed for the dusting experiment. The group on the east side of the entrance was dusted while that on the west remained as a check. The group dusted consisted of 270 stems while the check had 300.

The dust chosen was Niagara copper carbonate dust D-6. About one quart of the dust was employed during each application with a hand duster, the plan being to keep the leaves, stems and fruit covered with dust. Care was taken to dust under surfaces of leaves and fruits. The number of applications was reduced to a minimum as the autumn of 1929 was exceptionally dry for Massachusetts.

The first application was made the day following the appearance of the first diseased snowberry, September 2, 1929, and afterwards as follows: September 14, 28, and October 12 when the pink spore masses ceased to form on the snowberries.

Observations for the final results were taken on November 22, 1929, or when the leaves had fallen and the snowberries should be at their height of beauty. The apparently healthy and the diseased snowberries were counted on 200 racemes, which were well distributed among the dusted plants and the checks. The snowberries on each of 100 racemes on the dusted plants averaged: apparently healthy 3.7; diseased 2.7. While the checks showed: apparently healthy 1.6; diseased 2.2 snowberries per raceme. However, the fact is recognized that many of the diseased snowberries had fallen and many racemes on the dusted plants and on the checks were entirely without fruit. Yet, the apparently healthy berries on the dusted shrubs were more than twice the number on the controls, which would seem to be an important fact to recognize. Yet, when so many diseased fruits were internally infected by the fungus advancing from the stem into the fruit, spraying and dusting would only control the dissemi-

nation of the fungus, which probably accounts for the dusting benefits just mentioned.

Sporulating cankers were found on the stems in 1929; so it would seem advisable to spray in the springtime with a dormant spray such as lime-sulphur.

As the parasitic fungus is systemic, it would also seem advisable to remove all canes bearing diseased fruits by pruning them to the ground during the dormant season and burning the rubbish. Then, starting with new canes the following season, apply a dormant spray in the springtime and dust in the fall. However, diseased fruits and leaves should be gathered and burned and the soil around the shrubs cultivated.

As a sanitary precaution, apple trees in the immediate vicinity should be properly sprayed and all limb-cankers pruned or properly attended. If the beauty of snowberries is to be retained, pruning, dusting and sanitation should be practiced.

SUMMARY

1. An anthracnose of snowberries was prevalent in northeastern United States during 1928. The disease was widely disseminated and prevalent in the New England States during 1927, 1928, and 1929.

2. The injury to the snowberry plants was severe, as about 60 per cent of the snowberries were infected, causing them to drop prematurely and thereby much of the beauty and value of the shrub lost.

3. The disease was caused by a fungus:

A. Ascogenous or perfect stage: *Glomerella rufomaculans* (Berk.) Spauld. & Schrenk.

B. Conidial or imperfect stage: *Gloeosporium rufomaculans* (Berk.) Thüm. (*G. fructigenum* Berk.)

4. The infection first appeared during the autumn; conidia formed on acervuli and the fungus was disseminated. Under proper conditions, perithecia formed in snowberries and conidia formed in overwintered diseased snowberries during the following spring. It is believed that hyphae overwintered in the buds, advanced into the pith of the new wood, passed along the hollow stem, then outward to the bark, through the flower stalks and the fruits.

5. The fungus infected and formed viable conidia in each of snowberries, coralberries, apples, pears, quinces, tomatoes, bananas, grapes and peppers. It did not infect cranberries.

6. The following controls are suggested:

1. Observe sanitation by destroying diseased plant parts and cultivate the soil around the shrubs.

2. Prune the diseased twigs and stems at the ground, starting with new wood which should be cared for by applying a dormant spray of lime sulphur.

3. Spray apple trees in the vicinity and attend all cankers.

4. Apply copper carbonated dust, beginning at the first appearance of diseased snowberries in September and continuing to keep the snowberries and leaves covered with the dust until freezing weather or sporulation ceases on the snowberries in the open.

PART II

ALTERNARIOSE OF THE SNOWBERRY CAUSED BY ALTERNARIA SOLANI FORMA SYMPHORICARPI

Barrus and Horsfall (1) reported an *Alternaria* in the tissues of the snowberry and that it caused water-soaked lesions. They isolated the fungus from stems of the snowberry plant and inoculated by puncturing the fruit and produced the disease. However, the life history and species of the fungus remained unknown.

Young (11) listed an *Alternaria* on the snowberry and believed it to be a physiological form of *Alternaria tenuis* Nees, or species No. 1, as reported by Saccardo (6). Young inoculated wheat and oat seedlings with conidia of an *Alternaria* which he had isolated from the snowberry, obtained infection and assigned the fungus to his physiological form nine (P. F. 9). He also reported that this organism did not infect cabbage in the greenhouse.

In the autumn of 1928, many diseased snowberries (*Symphoricarpos albus* L.) Blake var. *laevigatus* (Fernald) Blake which were covered with dark hyphae and bore conidia of an *Alternaria* were observed at Amherst, Massachusetts. In 1929, the disease reappeared and experimentation was undertaken for the specific purpose of determining:

1. How may the symptoms of alternariose be distinguished from anthracnose?
2. How important is the disease?
3. What Latin binomial should be assigned to the fungus?
4. How may the disease be controlled?

DISTRIBUTION AND SYMPTOMS

Alternariose of the snowberry was very widely distributed during the autumns of 1928 and 1929. In 1928 it was located in several New England States and New York State. In 1929 this disease was more prevalent than during the preceding season when it was sparsely distributed on each shrub examined. During both years it was associated with anthracnose and as prevalent. Actual counts of diseased fruits, in October, showed about 25 per cent of the snowberries on the shrubs bore symptoms of alternariose.

Snowberries infected with alternariose changed from a white color to a buckthorn or a dresden-brown, but when old they were a mummy-brown while those lately infected with anthracnose were rufous or red instead of yellow. Furthermore, snowberries infected with alternariose generally became soft and watery and, upon long standing, were covered with grayish or olive-black mycelium while those infected with anthracnose were rufous, finally turning to a dead black, wrinkling and mummifying with the surface often remaining smooth and without mycelium. However, both fungi causing these two diseases were often cultured from the same diseased parts of fruits and twigs of the snowberry plant (PLATE 19, FIG. *B*, 1–5; FIG. *D*).

ISOLATIONS AND INOCULATIONS

Monosporous cultures were made from conidia of an *Alternaria* which had completely covered several snowberries with its mycelium (PLATE 19, FIG. *B*, 1–5). Healthy snowberries were inoculated by placing conidia and mycelium from a monosporous culture in punctures, on unpunctured surfaces, on the calyx ends and on broken pedicels. Likewise, other snowberries were inoculated with *Alternaria Brassicae* (Berk.) Sacc., which had been isolated from the leaves of common cabbage (*Brassica oleracea*

var. *capitata* L.) and with *Alternaria Solani* (Ellis & Mart.) Jones & Grout isolated from tomato leaves (*Lycopersicon esculentum* Mill.). Proper checks were prepared and the inoculated snowberries were incubated in Coplin breeding jars lined with damp filter paper and stored at 20° C. One series of these inoculations was made in October, and another in November, 1929.

Two weeks subsequent to the inoculations made in October, all the checks and snowberries inoculated with *A. Brassicae* were healthy. The punctured snowberries inoculated with *Alternaria* isolated from the snowberry and from the tomato were infected with an *Alternaria*, the inoculum from the tomato apparently being more active than that from the snowberry. When the inoculum was placed on the calyx end, only three of the 10 snowberries were infected; none of the snowberries with inoculum placed on the pedicels were infected. Thus the *Alternaria* parasitizing the snowberry and *A. Solani* which parasitized the tomato were the same physiologic form. However, the results of the November inoculations were somewhat confusing as so many of the snowberries apparently healthy were internally infected and this infection was apparent only when the snowberries were brought from the open and placed within a damp chamber stored in a warm room (20° C.). Even then, some of the snowberries which had remained healthy for several weeks suddenly showed infection. In one series, 40 apparently healthy snowberries were divided into four lots of 10 each and each lot treated as follows: Lot 1, check; Lot 2, inoculated with *A. Solani;* Lot 3, inoculated with *A. Brassicae;* Lot 4, incculated with a monosporous culture of an *Alternaria* from the snowberry. The surfaces of the snowberries were sterilized for 8 minutes in an aqueous solution of bichloride of mercury, 1 : 1000, to which had been added 2 cc. of HCl, washed in distilled water, inoculated and incubated in a sterilized Coplin breeding jar. After two weeks' incubation, each snowberry inoculated with an *Alternaria* from the tomato and from the snowberry was infected with an *Alternaria* which was sporulating. Three of the checks and four inoculated with *A. Brassicae* were healthy. However, snowberries in each series were infected with a *Gloeosporium* and one

or more in each series with an *Alternaria*. The cold weather in November had checked the activity of the pathogen in the open but its activity was resumed when the host parts were transferred to a growing temperature in the laboratory. Also, the *Alternaria* from tomato infected the snowberries, even though they were infected by anthracnose. However, from these inoculations it is to be noted that the *Alternaria* parasitizing the tomato and that parasitizing the snowberry are of the same physiological strain.

Leaves of potted Bonny Best tomato plants were inoculated with monosporous cultures of *Alternaria* isolated from the snowberry and from the tomato. Mycelium and conidia were spread on both upper and under leaf surfaces. Before inoculation, the soil in the pots was soaked with water and the plants atomized with distilled water. The inoculated plants were covered with bell jars lined with damp paper and stored in the greenhouse at a temperature averaging 24° C. Ten days afterward, the tomato leaves had been infected by conidia of *Alternaria* from both snowberry and tomato, while the checks were apparently healthy. Several of these lesions on the tomato plants bore the characteristic targeting and conidia of an *Alternaria*. This experiment was also duplicated by others (college students) with like results. These inoculations showed that the *Alternaria* parasitizing tomato leaves was the same physiologic form as that which parasitized snowberries.

Brown hyphae of an *Alternaria* were located in the palisade and mesophyll layers in the leaves of the snowberry plants and sometimes emerged through the stomata. However, the targeting which generally accompanies alternariose of leaves was not observed on leaves when collected in the open. When leaves of the snowberry plant were inoculated with the monosporous culture of *Alternaria* from the snowberry employing Clinton's method (**2**), they turned a dark gray color after three days' incubation and then the fungus seemed more active as a saprophyte on dead leaf tissues than as a parasite on the living host.

The *Alternaria* was also cultured from stems of shrubs. In July, 5 twigs which had borne diseased snowberries during the previous autumn were sterilized for 8 minutes in an aqueous

solution of bichloride of mercury (1 : 1000 with 2 cc. of HCl added), washed in distilled water, cross-sectioned through each node and internode of each year's growth and these sections incubated on potato dextrose agar. The results showed that over half of the sections contained hyphae of an *Alternaria*. The fungus was most prevalent in sections removed from the nodes and in the outer bark. Microscopic examination of free-hand sections adjacent to those which were incubated on the agar showed that *Alternaria* hyphae were under the bark in abundance and that they sometimes sporulated on the surfaces of both young and old canes. Furthermore, *Glomerella rufomaculans* was generally present in the cultures and the two fungi seemed very closely associated. No evidence is at hand to determine whether the *Alternaria* followed the path of penetration made by the *Glomerella* or vice versa or whether they were symbiotically associated.

During the winter of 1928–29, an *Alternaria* was cultured from the bark and from the buds of snowberry shrubs. These experiments seem to present evidence that the hyphae of the *Alternaria* were perennial in the bark and bud scales of the snowberry shrub and especially plentiful at the nodes.

MORPHOLOGY AND TAXONOMY

Fresh conidia were removed from naturally infected snowberries and measured by employing the proper technique for such work. The measurements in microns follow: with no crosswalls, 8.5 × 12 microns; 1 crosswall, 12 × 19; 2, 13 × 21; 3, 14 × 27; 4, 12 × 31; 5, 15 × 37; 6, 22 × 43; 7, 14 × 46, and with 8 crosswalls, 14 × 51 microns. Standard; 14 × 27 microns; 3 crosswalls; muriform; 72 per cent (100 measured and the pedicels excluded). Variation, 8.5–17 × 12–51 microns. Forms varying from clavate with 8 crosswalls and muriform to those globose with one cell (TEXT FIGURE 4, NUMBERS 3–11); pedicellate or non-pedicellate; pedicels 2 × 2–12 (standard) or sometimes 80 microns long; conidia catenulate, varying to the *Stemphylium* type (TEXT FIGURE 4, 1, 2, 10, 11). The conidia were slightly constricted at the crosswall when the spores were in chains and attached but decidedly constricted at the crosswalls when germinating

and somewhat constricted after having been submerged in water for some time. The colors of immature conidia varied from a buckthorn to a dresden brown while matured conidia were a mummy brown.

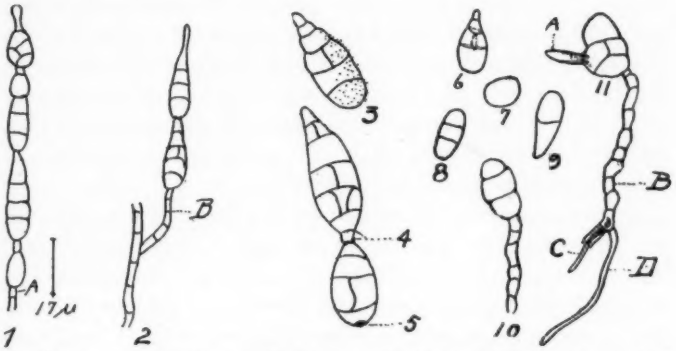

FIG. 4. Tracings from outlined, camera-lucida drawings showing various types of conidia and conidiophores of *Alternaria Solani* which parasitized the snowberry. No. 1–2. Chains of conidia as they formed in a drop slide cultures of carrot decoction which originally contained a single conidium removed from the snowberry. The *Alternaria* type of conidium (catenulate) is evident. No. 1 *A*, and No. 2*B*, the original or primary conidiophores; No. 3–10. Conidia removed from the snowberry; No. 3. A minutely spinescent conidium; No. 4–5. Large conidia; No. 7–10. Conidia bearing cells varying from 1 to 5 in number; No. 10. The *Stemphylium* type of conidium; No. 11. *A, C, D* Germtubes of a conidium which had been submerged in water for 20 hours. Cells of the pedicel (*B*) formed germtubes (*C* and *D*). Pedicellate cells did not "germinate" when old spore materials were employed.

Measurements of conidia of *Alternaria* from the snowberry and from the tomato as reported by several investigators:

	Hosts	
Investigator	Tomato	Snowberry
Young (4).....	7–20 × 14–60 microns	7–18 × 15–35 microns
	11–14 × 14–56	Pedicels included
	11–14 × 40–50	
Saccardo (3).....	Macrosp. tomato	*Alter. tenuis* (Young's)
	20–22 × 100–120	14–15 × 30–36
Rands (2).....	Same as on potato	
	12–20 × 120–296	
Davis.....	12–20 × 18–160	8.5–17 × 12–51
	Pedicels omitted (80 microns)	14 × 27 (standard)

Considerable variation in the sizes of conidia is to be noted in

the above table. However, mycologists generally concede that spore measurements are of little, if any, aid in determining a species of *Alternaria*. Young (**11**) reported conidia of an *Alternaria* parasitizing the snowberry as measuring 7–18 × 15–35 microns. He included the pedicels in his measurements. Saccardo (**3**) gave the conidial measurements of *A. tenuis*, of which Young believed the fungus on snowberry a physiological form, as 14–15 × 30–36 microns. He also described the conidia as muriform, 3–5-septate and constricted at the septa. Young tabulated measurements of *Alternaria* on *Lycopersicon* and a wide variation in sizes is to be noted.

The measurements of conidia from the snowberry as reported by the writer vary from those reported by Young and those reported by others for *A. Solani*. The environmental conditions play such an important part with the sizes of conidia that this variation is to be expected. These measurements of conidia do not vary sufficiently to exclude this *Alternaria* from *A. tenuis*, considering variation in the sizes reported by Young and Saccardo. However, these conidia are shorter, beaks less prominent and shorter than those of *A. Solani* as reported by Rands (**4**), who believed that *A. Solani* is the same species of *Alternaria* as that which parasitizes the Irish potato (*Solanum tuberosum* L.). The action of *A. Solani* and of the *Alternaria* on the snowberry was physiologically the same but, considering the sizes of the conidia and pedicels, the two fungi were morphologically different. In view of the fact that at the present time there is no accurate method of classifying an *Alternaria* by spore measurements, the physiology of the fungus is the more reliable. However, this fungus is a morphological variation and a physiological form of *A. Solani*. Perhaps many physiologic forms exist but, until a definite scheme of classification is presented to mycologists, all these classifications remain arbitrary. Since no perfect stage of the fungus was observed, a tentative classification is suggested for the organism causing the anthracnose of the snowberry: *Alternaria Solani* (Ellis & Mart.) Jones & Grout forma **Symphoricarpi** forma nov.

CONTROLS

On September 2, 1929, snowberry shrubs were dusted with Niagara copper carbonate dust D-6. The plants were afterwards dusted on September 14, 28, and October 12, or when the growing season had passed. The leaves, stems, and fruits were constantly covered with the dust during September and October. In November, when the berries should be at their height of beauty, observations were made and recorded. Only five snowberries with *Alternaria* could be found on the dusted plants while a good number were to be noticed on the undusted checks. As this fungus was so often associated with the *Glomerella rufomaculans* no definite check or counts could be obtained. However, dusting under the conditions observed checked the fungus. Since the fungus resides in the canes, all rubbish from pruning the shrubs should be burned together with the old diseased snowberries and the soil around the base of the shrub thoroughly tilled. As conidia of *Alternaria* overwinter in this latitude and viable mycelium was found in the bark under winter condition, it would seem advisable to employ a dormant, spring spray of lime sulphur.

SUMMARY

1. The symptoms of alternariose may be distinguished from anthracnose as follows: snowberries infected with alternariose were yellow or brown (buckthorn, dresden- or mummy-brown) color, while those with anthracnose were red or black (rufous to olive or dead black). A soft watery rot generally accompanied alternariose while a dry rot, wrinkling and mummifying of the snowberries accompanied alternariose.

2. Alternariose is important since it may cause 25 per cent of the berries to rot. It also parasitizes the bark of the cane and the bud scales.

3. The Latin binomial of the fungus is *Alternaria Solani* (Ellis & Mart.) Jones & Grout. This classification is principally based on its physiological behavior. However, if both the physiological and morphological characters are to be considered, *Alternaria Solani* (Ellis & Mart.) Jones & Grout f. (forma) *Symphoricarpi* is suggested.

4. The disease was controlled by keeping the stems, leaves, and snowberries covered with copper carbonated dust during September and October.

Pruning, sanitation, tillage of the soil around the shrubs and application of a dormant spray (lime sulphur) in the spring are suggested controls.

PART III

A BOTRYTIS ROT OF THE SNOWBERRY CAUSED BY BOTRYTIS VULGARIS

In October 1928, infected snowberries of a yellow-ochre color were observed on all snowberry shrubs inspected. As the cause for this yellowing of the fruit was unknown, ten of these yellow snowberries were placed in a Coplin breeding jar lined with damp filter paper and stored at room temperature. Three days afterwards, most of the snowberries were covered with a pale smoky-gray mycelium. After five days' incubation, conidiophores bearing several clusters of hyaline conidia of the *Botrytis* type appeared on the surfaces of several snowberries (PLATE 18, FIG. *A*, 3; PLATE 19, FIG. *B*, 11, 12, 13).

ISOLATION AND INOCULATION

The fungus was isolated by transferring conidia on conidiophores to potato-dextrose agar, employing the technique common to such procedure. Pure, sporulating cultures were readily obtained.

For inoculating purposes, forty snowberries were removed from a plant bearing a low percentage of infected fruit (50 per cent). The surfaces of these snowberries were sterilized for 8 minutes in an aqueous solution of bichloride of mercury, 1 : 1000, to which 2 cc. HCl had been added. The inoculum consisted of conidia and mycelium from pure cultures obtained by methods previously described. The snowberries were inoculated in four series of 10 each; series A, punctured and the inoculum inserted; series B, inoculum spread on the surface (sides); series C, inoculum spread on the calyx end; series D, checks consisting of five punctured and five unpunctured snowberries. The inoculated snowberries were then incubated in a Coplin breeding jar, lined with damp filter paper and stored at 18° C. (11–16–29).

Five days after the inoculation, each inoculated snowberry was entirely discolored by the *Botrytis*, having turned from its normal snow-white to an antimony yellow or yellow ochre. Pale smoky-gray floccose mycelium covered the surface of three snowberries and, on the next day, citron-drab to clove-brown conidiophores bearing aggregates of hyaline conidia of a *Botrytis* were observed.

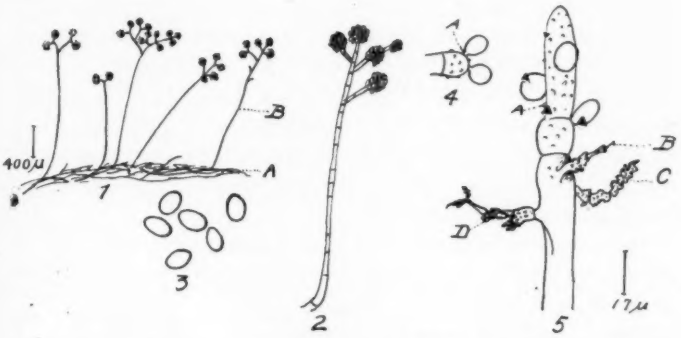

FIG. 5. Tracings from outlined, camera-lucida drawings of conidia and conidiophores of *Botrytis vulgaris* Fries, removed from snowberries. No. 1. Grape-like clusters of conidia on various types of branching conidiophores as observed on the snowberry; No. 2. Somewhat enlarged and diagrammatic representation or a conidiophore showing the cross-walls and enlarged base; No. 3. Various shapes of the conidia, nearly circular, elliptical and ovate in outline; No. 4. The end of a living conidiophore showing conidia attached by small projections representing sterigmata; No. 5. A conidiophore from which most of the viable conidia have been removed. The cells of the branches *B*, *C*, and *D* have collapsed after the conidia matured. The surface (*A*) is roughened by projections showing where conidia were attached.

Leaves of the snowberry plant were inoculated and, after three days, the fungus had turned them a dark-brown color and was sporulating abundantly on their surfaces. The fungus was also collected on leaves in the open. Diseased leaves were removed from the snowberry plant, stored in a damp chamber and, on several occasions, some showed symptoms of a *Botrytis* disease. Furthermore, the fungus sporulated abundantly on some leaf surfaces.

PATHOGEN

The conidiophores were mostly erect, averaged 2 mm. in length; 12 microns in diameter; 5-septate (3–15 septa); mostly dichotomously branched, 1 to 4 times (TEXT FIGURE 5, NUMBER 1); bore 1 to 12 clusters of conidia on short, obtuse or rounded, hyaline branches which soon shriveled (No. 5). Conidia were somewhat persistent, mostly on secondary conidiophores (branches); hyaline when young but tinged with fuscus when matured; globose to ovate or elliptical (No. 3); measurements in microns of 100 fresh conidia; limits, 6.5–14 × 8.5–19; standard, 9 × 14. Sterigmata, when present, averaging 1 × 3 microns (No. 4). No microconidia were observed. Sclerotia, in cultures, plentiful, nearly black, globose or cylindrical to flat, somewhat irregular in outline, 1 to 7 mm. When cultured, produced mycelium and conidiophores but no apothecia.

The above description compares favorably (**5**) with that of *Botrytis vulgaris* Fries, as recorded by Saccardo; more especially of the *Botrytis furcata* Fries type.

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EXPLANATION OF PLATES

PLATE 15

Photographs showing anthracnose and photomicrographs illustrating the epidermal cells of the snowberry. Materials used in Figs. *C*, *E*, and *F* were from the same snowberry and treated at the same time. Fig. *A*. Clusters from two different shrubs collected in November: 1, 2, Diseased snowberries with acervuli on the surface; 3, 4, Mummies or old, shriveled, diseased snowberries. Fig. *B*. Different stages in the development of anthracnose: 1, 2, 3, Initial symptoms of the disease, areas which have turned a rufous color; 4, 5, Diseased areas turned rufous color and mycelium on the surface; 6, 7, After 5 to 7 days, pinkish conidial masses indicated the position of acervuli and were characteristic of the disease; 8, A snowberry six days after inoculation by puncturing the epidermis at the side of the berry and inserting mycelium. Fig. *C*. Epidermal cells of a dried, healthy snowberry mounted in lactophenol for 8 weeks: 1, 2, 3, 5, Suberized protoplasmic membranes; 4, Cell walls. Fig. *D*. Epidermal cells removed near the calyx cup of a young snowberry; mounted in lactophenol for 48 hours: 1, A stoma with chlorophyll in the guard cells; 2, Suberin on the upper and side walls. Fig. *E*. Cells boiled in a 1 per cent potash solution for 15 minutes removed after 15 hours and mounted in lactophenol: 1, Cell wall; 2, 3, 4, The cells now contain globules of suberin material somewhat oily in appearance. Fig. *F*. Suberin granules after the epidermal cell walls were removed by boiling in a 10 per cent solution of sulfuric acid and mounted in lactophenol: 1, Suberin granules at the side of the cells; particles are also to be seen in the center of the cell lumen; 2, Suberin particles on the side and near the floor of the cell.

PLATE 16

Photographs of snowberries showing symptoms of anthracnose; also, photomicrographs of acervuli and conidia of *Gloeosporium rufomaculans*. Fig. *A*. These snowberries had been collected and stored in a damp chamber overnight before photographed: 1, 2, 4, 14, 16, Early stages of infection; 6, 12, 18, 19, Mummies from which perithecia form; 5, 7, 8, and others show the fruiting stage; spore masses on the surface; 3, 5, 9, 17, Early stages in the wrinkling of infected snowberries. Fig. *B*. Photomicrographs of an exceptionally large young acervulus which had been triple stained and permanently mounted: 1 and 2, Cutin which has been raised by the hyphae; 3, Conidium on its conidiophore in an acervulus; 4, 7, 10, Hyphae of the fungus; 5, Wall of an epidermal cell; 6, Wall of a mesocarp cell. Fig. *C*. An acervulus one week old. Prepared as in Fig. *B*. (Part of the background in the negative was opaqued): 1, Deliquescing hyphae; 2, Conidia in gelatinous material; 3, Subcutaneous hyphae. Fig. *D*. Photomicrograph showing different sizes, shapes and contents of conidia.

PLATE 17

Photomicrographs of perithecia which had been removed from snowberries and mounted in green lactophenol. Fig. *A*. Perithecia separated by dissection: 1, 2, 3, Beaks of perithecia; 4, 5, 6, "Hairs." Fig. *B*. Perithecia on the stroma: 1, The floccose stroma with perithecia in place; 2, Ascospores

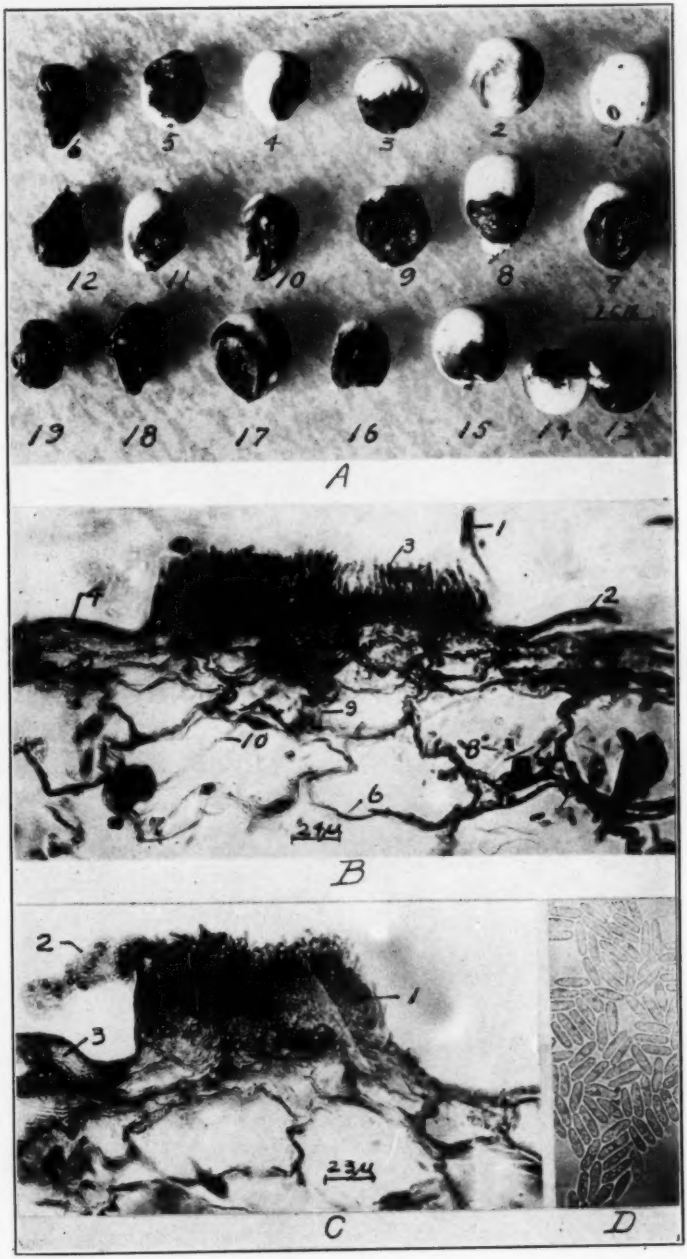
escaping through the pore of a perithecium. Fig. *C*. Hand sections of perithecia near the outer walls: 1, Loose hyphae on the outer walls; 2, Floccose hyphae lining the inner walls; 3, Thick-walled hyphae composing the middle wall; 4, Floccose stroma. Fig. *D*. Hand sections of a perithecium showing asci in place.

PLATE 18

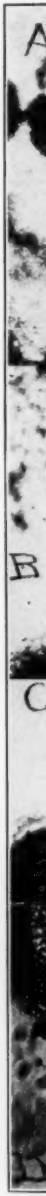
Photographs showing infected snowberries bearing fruiting organs of several fungi. Also, the structure of an ascus and ascospores of *Glomerella rufomaculans*. Fig. *A*. Nos. 4, 8, Snowberries infected by *Penicillium* sp.; 3, 7, Infected with *Botrytis vulgaris;* 1, 2, 5, 6, Infected by *Gloeosporium rufomaculans*. Fig. *B*. Perithecia on snowberries: 1–6, Inoculated with a pure culture and incubated in a moist chamber 21 days at 20° C.; 7–9, Diseased snowberries collected in the field and incubated as in Nos. 1–6 above. Fig. *C*. An enlargement of Nos. 2 and 5 in Fig. *B*: 1, Perithecia with beaks protruding; 2, Mycelium with conidia; 3, Immature perithecia. Fig. *D*. The contents of an ascus mounted in green lactophenol: 1–5, Asci containing ascospores; 6–7, Two ascospores. Fig. *E*. Photomicrograph of an ascus showing in *F* which has been traced with ink: 1, Foot of the ascus; 2, One of the allantoid ascospores which has germinated within the ascus. Fig. *F*. Photomicrograph of the ascus traced in Fig. *E*.

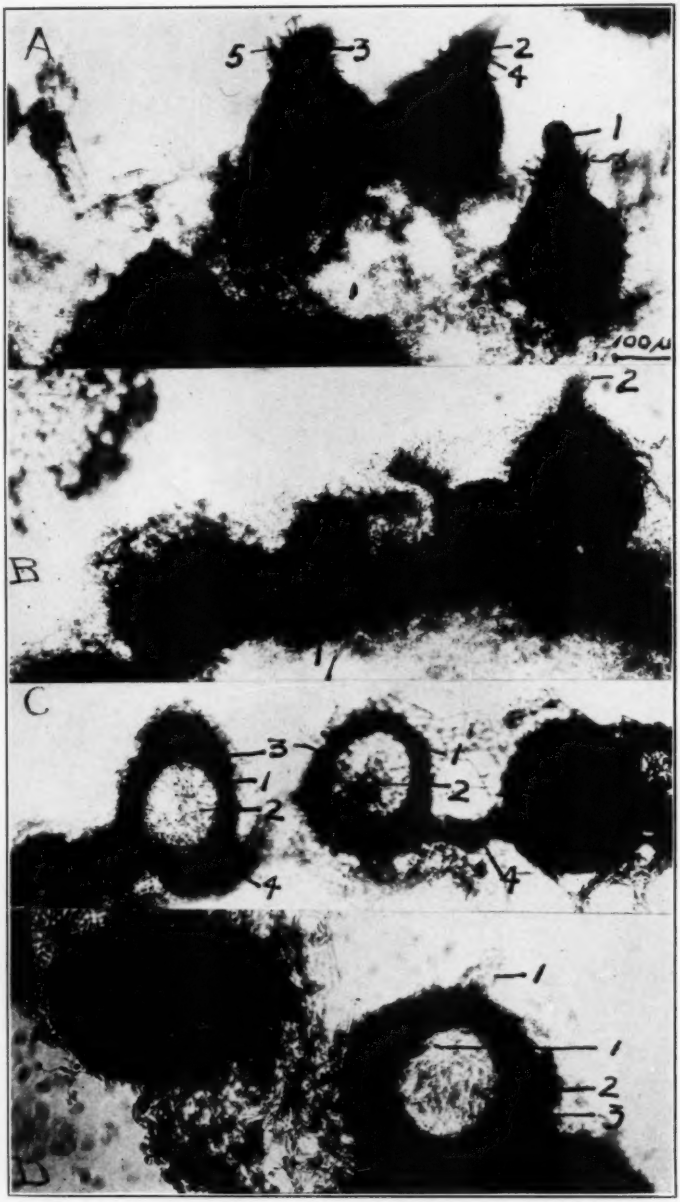
PLATE 19

Photomicrographs of the external layers in the fruit and bark of snowberry plants together with the symptoms when the fruit is infected by *Gloeosporium*, *Alternaria* and *Botrytis*. The materials were hand-sectioned and mounted in green lactophenol for one month before photographing. Fig. *A*. Mycelium of *Glomerella rufomaculans* within the epidermal cells of an infected snowberry: 1, 2, 3, Hyphae passing through pits in the cell walls; 4, 5, 6, 7, Hyphae engulfing nuclei. Fig. *B*. Two twigs bearing snowberries infected with *Gloeosporium*, *Alternaria*, and *Botrytis* (10–21–29): 1–5, Snowberries infected with *Alternaria Solani* forma *Symphoricarpi;* 7, 8, 9, Snowberries infected with *Gloeosporium rufomaculans;* 10, 11, 12, Snowberries infected with *Botrytis vulgaris;* 13, Leaf of a snowberry plant infected with *B. vulgaris*. Fig. *C*. Perithecia and epidermis removed from a snowberry which had been collected from a twig during October. These materials had been mounted for three weeks in green lactophenol, and a cover glass pressed on them so as to cause the contents to emerge: 1, 2, 3, Perithecia with pores; 4, 5, 7, 8, Ascospores; 6, Epidermal cells of the snowberry. Fig. *D*. Outer bark from a diseased twig of a snowberry plant infected with *Alternaria Solani:* 1, Mycelium on the surface; 2, 3, Cells of the mycelium forming rounded fuscous bodies analogous to chlamydospores; 4, 5, Conidiophores; 6–9, Mycelium within stem tissues.



ANTHRACNOSE OF THE SNOWBERRY


A
B
C



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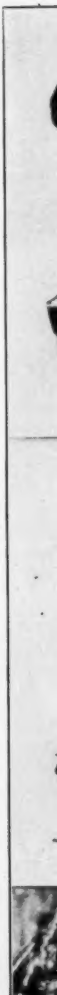
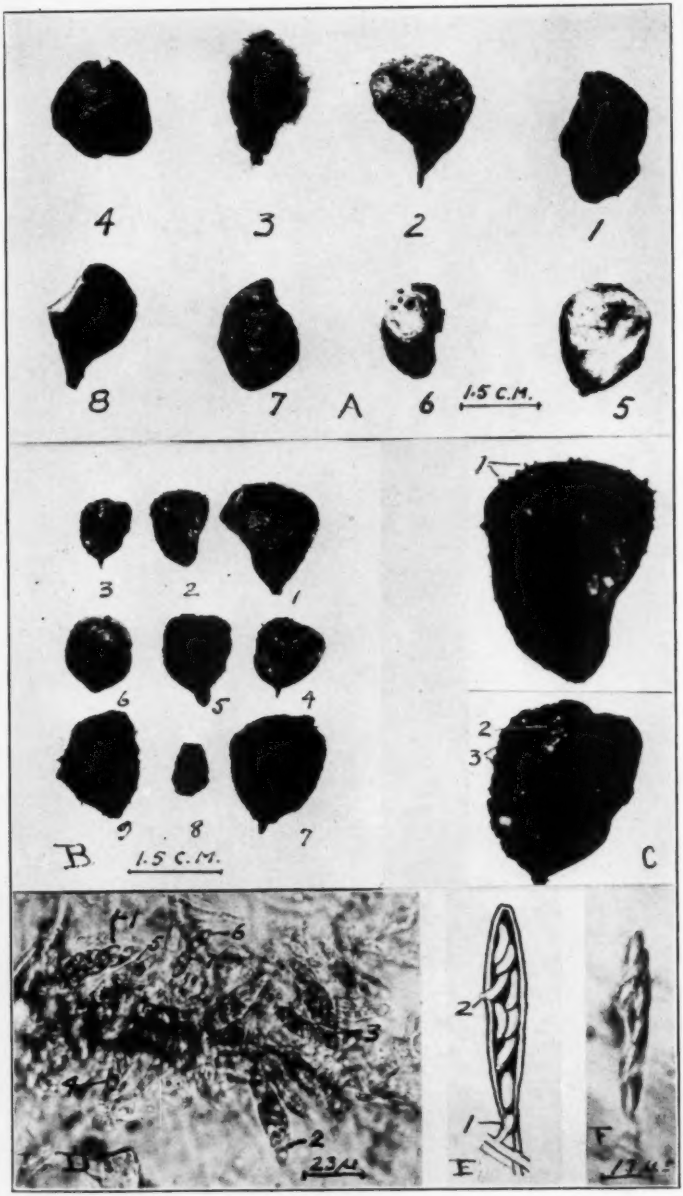
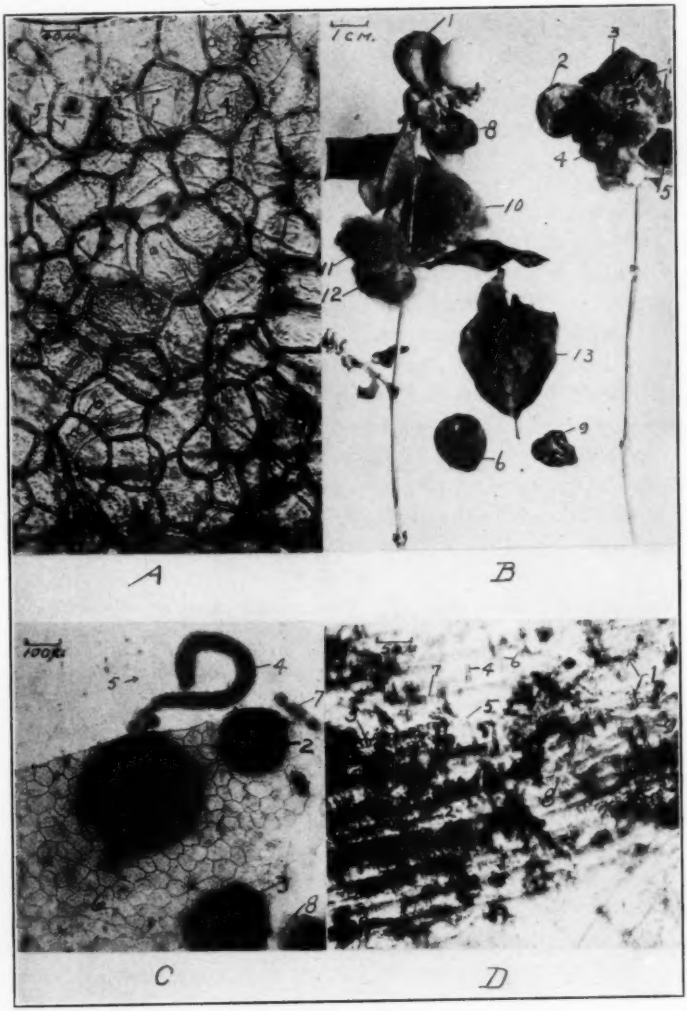




ANTHRACNOSE OF THE SNOWBERRY



ANTHRACNOSE OF THE SNOWBERRY

OBSERVATIONS ON PYTHIUM DICTYOSPORUM

FREDERICK K. SPARROW, JR.

(WITH PLATE 20 AND 1 TEXT FIGURE)

Although other members of the genus *Pythium* have in many instances been studied at length, *P. dictyosporum* of Raciborski has received but brief attention since in 1891 it was first reported by him parasitic in *Spirogyra insignis* (Hass.) Kütz., at Cracow, Poland (**6**). The following year in a paper [1] in Polish he described the fungus more fully and illustrated various stages in the life history of the organism (**7**), which he then stated was found parasitic in *Spirogyra nitida* (Dillw.) Link. In 1895 under the name *Nematosporangium dictyosporum* (Racib.) Schröter, de Wildeman reported the fungus from France in an unnamed species of *Spirogyra* (**10**). So far as the writer has been able to ascertain, these are the only published records of the occurrence of the fungus. In October, 1926, however, in a laboratory culture of *Spirogyra crassa* Kütz., collected at Belmont, Mass., the present writer found within this alga the golden reticulate oöspores (FIG. 1, *A*, *B*) and, later, the sporangia of this fungus. Additional knowledge of the life history and morphology of *P. dictyosporum*, resulting from a study of the fungus as found in this algal culture, as well as under conditions of pure culture, is presented in the following paper.

METHODS

By micro-manipulation with fine platinum needles, oöspores with attendant mycelium as well as oöspores alone were separated from the infected algal filaments, that had been washed in sterile water, and were planted in petri dishes of nutrient agar. Colonies of the fungus were readily secured in this manner and by repeated transference bacteria-free cultures were obtained. Non-sexual reproduction was induced in pure culture by thoroughly

[1] The writer wishes to express his thanks to Professor Leo Wiener of Harvard University for his kindness in translating portions of Raciborski's paper.

washing mats of mycelium grown in pea or bean broth and transferring them to sterile distilled water. At room temperature (21° C.), mycelia usually produced zoöspores in 10–15 hours after this treatment.

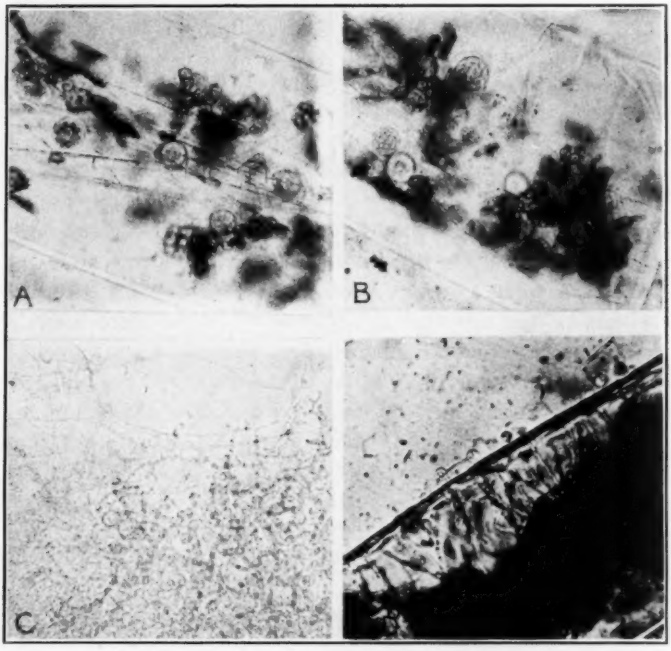


FIG. 1. *A*, General habit of the fungus within the *Spirogyra* cell. Several o spores are shown. × 550; *B*, Similar to the preceding, showing more clearly the character of the o spore walls. × 550; *C*, Mycelium of the fungus which has been grown in pea broth, ten days after its transference to distilled water. The distorted condition of the hyphae may be appreciated by compairing them with the typical, slender ones near the top of the photograph. × 500; *D*, A cell of *Spirogyra* which has been penetrated by the germ tubes of a number of cystospores, the empty shells of which may be seen adhering to the outer wall of the alga. × 550. Reduced one third.

MORPHOLOGY AND DEVELOPMENT

Within the *Spirogyra*, the fungus formed a non-septate mycelium which perforated the end walls and occasionally sent out

branches even through the lateral walls of the host. The hyphae were isodiametric, hyaline, and finely granular, averaging 1.8 μ and seldom being over 2 μ in diameter. In pure culture on various types of media, both liquid and solid, the branching was more profuse. A slight increase in diameter was also noted, the filaments generally attaining 2.5–3.0 μ, save on 1 per cent proteose-peptone agar, where they frequently reached 5.4 μ.

Several specialized vegetative structures not reported by either Raciborski or de Wildeman were observed under certain conditions in this species. For example, the mycelium, when grown on 5 per cent maize oil agar and in such solutions of proteins as 1 per cent peptone or 1 per cent proteose-peptone, was characterized after several weeks by the formation of sub-spherical terminal or intercalary hyphal swellings at frequent intervals along its filaments (PLATE 20, *D*, *E*, *F*). These bodies, which were not cut off by septa from the hyphae, possessed a densely granular content and numerous refractive granules which turned orange when stained with Sudan III.

Moreover, appressoria were also found to develop. These structures did not occur in *Spirogyra*, nor had they been seen by Raciborski and de Wildeman. However, during the course of a series of inoculation experiments, to be described in a later paper, they were found to be formed when the mycelium came into contact with the walls of *Tolypothrix* sp. (?) and *Rhizoclonium hiergolyphicum* (Ag.) Kütz. (PLATE 20, *B*, *C*). The tip of the hypha, coming into contact with a cell wall of the alga, adheres to it by means of a refractive, possibly mucilaginous, substance, slowly becomes distended, and forms a somewhat pyriform structure. This appressorium is securely anchored to the algal cell wall by a disk of cementing substance which is often easily visible. Penetration of the cell wall is accomplished by means of a slender refractive tube produced at the tip of the appressorium. After penetration, there is formed at the distal end of this tube a hypha of ordinary diameter, which by further growth and branching establishes itself within the host cell (PLATE 20, *B*).

One further point regarding the vegetative thallus of this fungus seems worthy of mention. Bits of mycelial mats, grown in some liquid medium, when placed without washing in capsule

dishes and covered with sterile distilled water, after 7–10 days, showed greatly swollen and distorted hyphae (FIG. 1, *C*). These were formed by the slow accumulation of protoplasm within certain hyphal regions and by the consequent distention of these parts. They did not seem to perform any particular function, although they were observed over a period of ten days. In certain closely allied species, under conditions favorable for non-sexual reproduction, similar appearing bodies are formed as a sub-basal part of the filamentous sporangium. It is of interest to note the absence of such reproductive activity in this instance.

NON-SEXUAL REPRODUCTION

The sporangia of *P. dictyosporum* are not differentiated from the vegetative hyphae, but are merely slender branches of the extramatrical mycelium which, with little or no constriction in diameter, grow out through the lateral walls of the host (PLATE 20, *A*).

The successive changes which culminate in the production of the zoöspores in this species do not differ materially from those to be described in some detail for a closely related species in a forthcoming paper. They conform, in the main, to Raciborski's meagre account, as well as to the more lengthy descriptions of Ward (**8**) and Butler (**1**) for several congeneric forms, and hence, save for one detail, will be treated very briefly here.

Little information is available regarding the rather critical changes occurring in what has been termed the "sub-apical" region of the sporangium, immediately preceding the formation of the vesicle. This has possibly been due either to the rapidity with which these stages occur, or to the fact that attention has been fixed on the more obvious enlargement of the brightly glistening apical material.

The appearance of the tip of the sporangium a few seconds before the emergence of its protoplasm is shown in Plate 20, *G*. It may be seen (in optical section) that the apex is divided into three definite regions: an upper, very refractive, crescent-shaped one; immediately below this, a slightly less hyaline zone; beneath this, a pale, slightly refractive, very finely granular region. The distal end of the latter zone is crateriform, and bears at its apex

a highly refractive bit of material which suggests a pore (PLATE 20, *G*). The protoplasm becomes more densely granular a short distance below the tip. If conditions are favorable for the process, there ensue, in rapid succession, the stages seen in Plate 20, *H*, *I*, *J*. In Plate 20, *H*, the refractive dome has lost its double contour, and has commenced to enlarge, and the expansion of the finely granular, crateriform protoplasm distally has been initiated. No change in the position of the lower, more densely granular material has occurred. This stage lasts only an instant and a third stage immediately ensues (PLATE 20, *I*). Here the hyaline cap has continued to expand, the cone of pale protoplasm has become even more distended, and its refractive "pore" has apparently burst or deliquesced, resulting in the liberation into the vesicle of a very finely granular material. The lower, more granular protoplasm has now started to move toward the tip of the sporangium where it will be discharged into the enlarging vesicle (PLATE 20, *J*). The flow of protoplasm thus initiated continues at a fairly even rate until the contents of the concomitant hyphae are exhausted. Cleavage of the homogeneous protoplasmic mass is then initiated, and the subsequent maturation processes result in the production of a variable number of zoöspores in the vesicle (PLATE 20, *L*). When maturing under somewhat foul conditions, the vesicular protoplasm may become quite vacuolate (PLATE 20, *K*).

Thus it may be seen that the finely granular, somewhat hyaline protoplasm with its crateriform apex appears to play an intimate part in the initiation of the ejection of the protoplasm from the tip of the sporangium. That it is of a somewhat different material from the underlying, coarse substance filling the rest of the sporangium is apparent not only from its distinctive physical properties, such as refractivity and expansive power, but from its greater affinity for such stains as muchaematein, ruthenium red, and Bismark brown.

Raciborski (**7**) indicated that there were four zoöspores produced by each sporangium of this species, while de Wildeman (**10**) gave the number as four, followed by a question mark. In the American material, four swarmers were sometimes observed in a single vesicle, but they were by no means restricted

to this number, more than forty being counted in some instances (PLATE 20, *L*). As the number of zoöspores produced in a single vesicle depends upon the amount of protoplasm contained between the delimiting hyphal septa, it appears that Raciborski's fungus differed from the writer's and from all other described species of *Pythium* in the regular intervals at which these walls must have occurred.

The zoöspore of *Pythium dictyosporum* is of the laterally biciliate type. Its body, which is 9.0 μ long by 5.4 μ wide, is not quite so rotund as that described by Weston (**9**) for the zoöspore of *Thraustotheca*, but otherwise the two are similar in general appearance and motility (PLATE 20, *M*). The course taken by the actively moving swarmer has invariably been described by investigators working on similar organisms as spiral, whereas the path actually produced is more accurately described as a helicoid one. After a period of motility, the length of which depends upon the conditions of the surrounding medium, the zoöspore comes to rest, its cilia are absorbed (PLATE 20, *N*), and a promycelium is produced (PLATE 20, *O*). Repeated swarming was observed by Raciborski but not by the writer.

The actual penetration of the algal filaments is accomplished in most cases by the zoöspore. This body, upon coming into contact with the wall of the *Spirogyra*, becomes quiescent, loses its cilia, and assumes a spherical shape. The body thus formed has been termed by Weston (**9**), in the case of *Thraustotheca*, a "cystospore," a name which is also convenient to use in the fungus herein described. These bodies, in *Pythium dictyosporum*, are from 8.5–10 μ in diameter. From the surface of the spore in contact with the wall of the *Spirogyra*, a fine, hyaline, cylindrical tube is produced, which, as it elongates, penetrates the cell wall of the host. The encysted zoöspore is apparently cemented to the wall of the alga before the development of the penetration tube, for zoöspores at this stage cannot be separated from the *Spirogyra* filament when the latter is vigorously shaken in water.

After penetration, the content of the zoöspore passes through the tube at an imperceptible rate and emerges, surrounded by a wall, into the interior of the *Spirogyra*, the empty cystospore

being left adherent to the outer wall (FIG. 1, *D*). Once inside the host cell, the advancing germ tube assumes the diameter of an ordinary hypha and, without branching, grows forward, apparently seeking the food afforded by the chloroplast. Further growth is characterized by ramification of the hyphae throughout the algal cell and penetration of the end walls of the host, with subsequent infection of the whole filament after four or five days.

SEXUAL REPRODUCTION

The sexual reproduction of *P. dictyosporum*, because of the unique structure of its oöspore, is of unusual interest both from the phylogenetic and ontogenetic points of view. The occurrence here, in one of the more "primitive" species of the genus, of an oöspore, the structure of which approaches the complexity attained by higher members of the Peronosporales, has been unrecognized by those who attach phylogenetic importance to this organ.

Raciborski's account of the sexual reproduction is, on the whole, quite complete, although certain structural details of the oöspore, as well as measurements of the sex organs, seem to have been omitted. The formation of the oögonia and antheridia presents no features of interest, being similar to that found in other species of *Pythium*. The antheridium is of the "crook-neck," clavate type, being, when fully mature, about 20 μ long by 8 μ wide (PLATE 20, *P*). Raciborski states that there may be one or two of these, either androgynous or of diclinous origin, attached to each oögonium. In the American material but a single antheridium, of diclinous origin, was found on each oögonium. The separation of the antheridial protoplasm into a hyaline peripheral layer and a central coarsely granular portion, the formation of a narrow fertilization tube through which a part of the inner "gonoplasm" is discharged into the oögonium (PLATE 20, *P*), and the subsequent maturation of the egg thus fertilized, closely follow Raciborski's description. Only a small portion of the antheridial content seems necessary for fertilization, as no empty antheridia were observed adhering to maturing oöspores, nor were any figured by Raciborski. According to the latter, the process of fertilization required about two minutes.

The oögonium when fully formed is about 21.5 μ in diameter and is ordinarily terminal in its position. Coincident with the formation of the fertilization tube by the antheridium, the oögonial content contracts somewhat and there is differentiated an outer, narrow layer of pale periplasm and an inner, more dense gonoplasm (PLATE 20, *P*). After fertilization, a series of regularly disposed vacuoles appears in the now coarser periplasm (PLATE 20, *Q*). These enlarge and become more angular (PLATE 20, *R*), indicating the initiation of coalescence of the intervacuolar material. Owing to its being mounted in glycerine, the whole content of the maturing oöspore shown in Plate 2, fig. *R*, has slightlv contracted away from a thin wall which probably soon after fertilization has been formed around the periplasm, but which, because of its tenuity, escaped detection. The intervacuolar material continues to coalesce (PLATE 20, *S*), and, with the exception of its midregions, falls away from the periplasmic wall (PLATE 20, *T*), deliniating the reticulations characteristic of the mature oöspore. Coincident with the aforementioned changes, a narrow wall is laid down around the gonoplasm which now is greatly condensed (PLATE 20, *T*).

The mature oöspore lies loosely within the old oögonial wall. The thick integument formed from the intervacuolar material of the periplasm is now a golden color and is raised in a series of reticulations that at their junctures form acuminations, the apices of which are generally in contact with the persistent outer periplasmic wall (PLATE 20, *U*). The regions formerly occupied by vacuoles now contain a homogeneous substance of a very low refractive power, probably derived from the contents of these vacuoles (PLATE 20, *U*). When oöspores are dried on slides, this material, together with the periplasmic wall, collapses upon the reticulations, but resumes its former position when water is added. This suggests that the substance is of a gel-like nature, which affords protection for the spore against excessive drought. Both periplasmic wall and gelatinous substance remain ensconced around the oöspore long after the oögonial and host walls have disintegrated. While several of Raciborski's figures plainly show this enveloping substance and its outer wall, there seems to be no reference to them in the text. Due to

de Wildeman's extremely poor figure of the oöspore, it is impossible to say whether or not they were present in his fungus. The content of the oöspore, surrounded by a moderately thick endospore wall, is similar to other species of *Pythium*, possessing the large central oil globule and the laterally placed, lenticular nucleus (PLATE 20, *T*, *V*).

Occasionally, oöspores were observed in which the fertilization tube had persisted after the antheridium and even the host cell had disintegrated. In one case, shown in Plate 20, fig. *V*, this tube appeared to be continuous with the reticulate wall of the oöspore.

So far as could be ascertained from Raciborski's paper, no measurements of the oöspore were given. In the form found by de Wildeman they were stated as being 14 μ in diameter. In the present material, they varied from 9–23.5 μ, the majority measuring 17.5 μ. Both extremes in size were often found in the same algal cell.

Germination of the oöspores was not observed by the writer or by de Wildeman, although Raciborski obtained it in a few cases after the spores had remained over four months (January to May) in water. The process took place very rapidly and involved the disappearance of the oil globule and the production of a filament which pierced the center of one of the polygonal areas, developed into a sporangium, and formed four zoöspores in the usual manner at its tip.

Inasmuch as the detailed description of *Pythium dictyosporum* Racib. is written in Polish, and hence generally unavailable, and as certain points brought out in this present study show a slight emendation of this species is necessary, it has been thought justifiable to include here a technical description of this form.

PYTHIUM DICTYOSPORUM [2] Raciborski.

Bull. Acad. Int. Sci. Cracovie **1891**: 283–287.

Kraków Nakt. Akad. Umiejet **24**: 1–9, 1 pl. 1892.

Nematosporangium dictyosporum (Racib.) Schröter, de Wildeman, Notes Mycologiques VI, Ann. Soc. Belge Microsc. **19**: 210–215, pl. 7, figs. 4–14. 1895.

[2] It should be noted that the specific name of this fungus is *dictyosporum* and not *dictyospermum*, as A. Fischer erroneously termed it and as has been perpetuated by practically all later writers.

Mycelium intra- and extramatrical, the latter forming, upon occasion, small, pyriform appressoria; composed of hyphae 1.8–2.0 μ in diameter, isodiametric, forming in certain media (generally protein) irregular swellings. Zoösporangia undifferentiated from the vegetative hyphae; zoöspores few to many (4 to 40) formed in a vesicle; of the laterally biciliate type, 9.0 μ long by 5.4 μ at their greatest width, coming to rest and rounding off into a cystospore, averaging around 10 μ in diameter; germinating by one or two germ tubes. Oögonia (in *Spirogyra crassa*) 12.6–28.8 μ (average 21.6 μ) in diameter. Antheridium of the crookneck type, 20 μ long by 8.0 μ at its greatest width, cut off by a single basal septum, its abruptly tapering apex making narrow contact with the oögonium; forming a fertilization tube of varying length, approximately 2.0 μ in diameter; one (rarely two) to an oögonium, borne terminally on a lateral branch of a hypha, usually distinct from the oögonial hypha. Oöspores (in *Spirogyra crassa*) one to an oögonium, not filling the oögonium, containing a single oil globule of variable size, surrounded by a smooth endospore wall; possessing a golden exospore wall which is raised to form reticulations, the junctures of which are elevated to form acuminate protuberances, adnate to a slender, persistent, periplasmic wall which encloses a gel-like substance of low refractive power in which the whole oöspore is imbedded; germinating after a period of rest (four months in water) by means of a single hypha which pierces the center of one of the polygonal areas bordered by the reticulations of the exospore, grows to a variable length, and becomes converted into a sporangium, and produces at its tip four zoöspores.

Parasitic in *Spirogyra nitida*, Cracow, Poland.

Parasitic in *Spirogyra* sp. (?), France, de Wildeman.

Parasitic in *Spirogyra crassa*, Belmont, Mass., the writer. (On artificial inoculation found capable of attacking also *Tolypothrix* sp. (?), *Rhizoclonium hieroglyphicum* and *Cucumis sativus*, as will be described in a later paper.)

DISCUSSION

Several points of interest are presented by this study of *Pythium dictyosporum*.

Thus far, all efforts to obtain sexual reproduction in this species on artificial media or in *Spirogyra* inoculated with zoöspores and mycelium from pure cultures have been unsuccessful. This is particularly unfortunate, as a cytological

study of this fungus would be of great interest, especially when compared with such forms as *Albugo* and *Peronospora*. However, work on this point is being continued. The methods used by Klebs (**5**) and Kauffman (**3**) to induce sexual reproduction in members of the Saprolegniaceae and those employed by Johann (**2**) in the case of *Pythium arrhenomanes* Drechsler were tried, but yielded no results. As oöspores, with no attendant mycelium, were used in obtaining some of the original cultures of the fungus, it is not probable that a single strain of a heterothallic form has been kept in cultivation.

There are, in the oöspore of this species, three well defined walls: an innermost one, surrounding the living contents; an outer, thicker layer, raised in a reticulate manner; and an outermost thin, persistent one surrounding the whole. Until detailed cytological evidence is forthcoming on their origin and development, they may be regarded (passing from the innermost to the periphery) as (*a*) the endospore wall of one layer, probably derived entirely from gonoplasm, (*b*) the reticulate exospore wall, (*c*) the outer, smooth, narrow periplasmic wall. The last two are unquestionably derived from periplasm.

King (**4**), defining the position among the Phycomycetes of *Araiospora*, a member of the Leptomitaceae, called attention to the oögonial origin of the fertilization tube of that fungus. He further pointed out that in *Albugo*, a member of the Peronosporaceae, this structure is formed not only by the antheridium, but, in some instances, partially by the oöplasm. The same writer also emphasized the persistence of the fertilization tube in *Albugo*. Taking *Araiospora* and *Albugo* as end points in a phylogenetic series, King suggested that *Pythium*, with its poorly developed evanescent tubes, "tends in a measure to bridge over the wide gap between them." It has been shown in *Pythium dictyosporum* that, in many instances, the fertilization tube is quite persistent and at times appears to be reinforced by oöplasmic material.

Without entering into a discussion as to the relative significance from a phylogenetic standpoint of the sexual and non-sexual structures of *Pythium*, the writer merely wishes to emphasize the fact that, in *Aphragmium*, the sub-genus containing the

supposedly more primitive species of the genus, there is known at least one form, *P. dictyosporum*, which possesses an oöspore comparable to those of certain of the higher Peronosporaceae.

SUMMARY

The present paper describes the finding for the first time in the United States of *Pythium dictyosporum*, a seemingly rare and little known phycomycetous parasite of *Spirogyra crassa* Kütz., the material of which was collected October, 1926, in a small pond near Belmont, Mass.

Details of the less known phases of the morphology and development of the fungus are given, especially with respect to certain critical stages in the formation of the vesicle, the method of penetration of the zoöspore, and the structure of the mature oöspore.

A brief consideration of the lack of sexual reproduction in pure culture, the identity of the various walls of the oöspore, the persistence of the fertilization tube, as well as a slightly emended technical description of the fungus, are included.

The writer wishes to express his appreciation for the aid and criticism given him by Professor W. H. Weston, Jr., under whose guidance the work reported in this paper was done.

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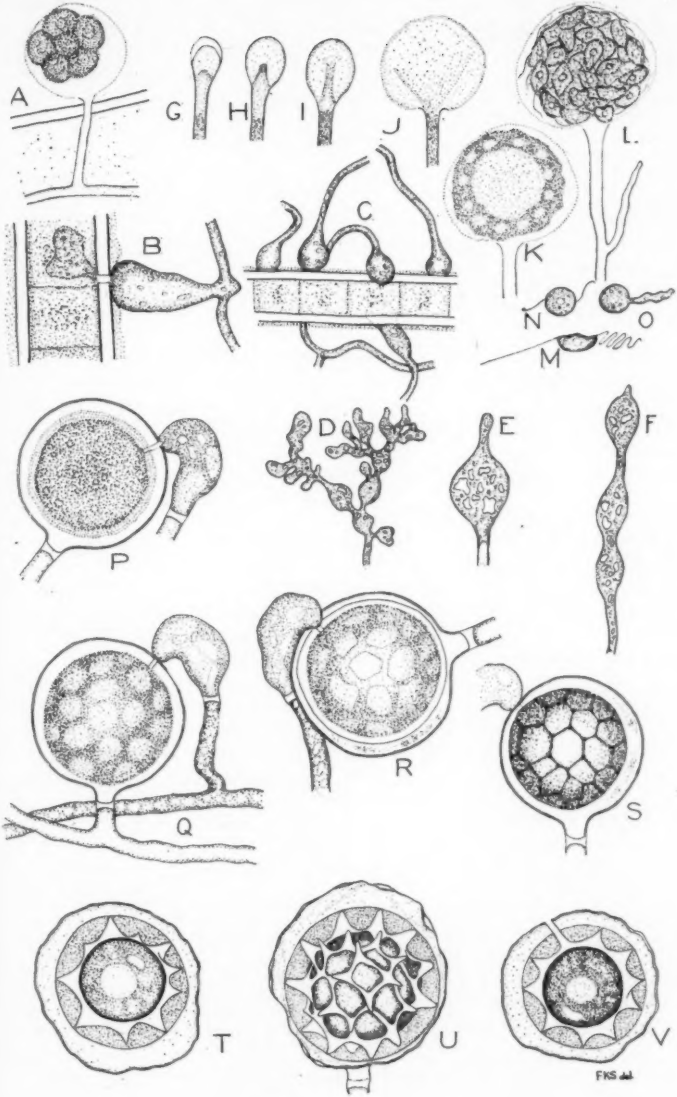
8. **Ward, H. M.** Observations on the genus *Pythium* (Pringsh.), Quart. Journ. Micros. Sci. **23**: 485–515, pls. 34–36. 1883.



PYTHIUM DICTYOSPORUM

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10. **Wildeman, E. de.** Notes Mycologiques VI, Ann. Soc. Belge Microsc. **19**: 210–215, pl. 7, figs. 4–14. 1895.

EXPLANATION OF PLATE 20

All the illustrations are from living material except Text Fig. 1, *A*, *B*, and Plate 20, *R*, which are from material mounted in eosine and glycerine. The drawings were made with the aid of the camera lucida. The magnifications given refer to the original plates which, for purposes of publication, have been reduced approximately one-third.

Fig. *A*. Filamentous sporangium which has emerged through the cell wall of the *Spirogyra* and within the vesicle of which zoöspores are being formed. × 1500.

Fig. *B*. Appressorium attached to the wall of *Tolypothrix*, showing the penetration tube and the protoplasm of the fungus which has passed through this channel into the host cell. × 2200.

Fig. *C*. A group of appressoria attached to a filament of *Tolypothrix*. × 1400.

Figs. *D*, *E*, *F*. Hyphal swellings formed by the mycelium in protein media. Fig. *D*, × 800; Figs. *E*, *F*, × 1400.

Figs. *G*, *H*, *I*, *J*. Stages in the formation of the vesicle and initiation of the egress of the sporangial protoplasm. × 1400.

Fig. *K*. Vacuolate condition of the maturing protoplasm within the vesicle; characteristically found under foul conditions of environment. × 1400.

Fig. *L*. Nearly mature zoöspores within the vesicle. × 1400.

Fig. *M*. Zoöspore in side view. × 1400.

Fig. *N*. Quiescent zoöspore retracting its cilia and becoming modified into the cystospore. × 1400.

Fig. *O*. Germinating cystospore. × 1400.

Fig. *P*. Optical section of the fertilized egg, showing the differentiation of its content into periplasm and gonoplasm. The antheridial protoplasm may also be seen to be of two types. × 2200.

Fig. *Q*. Fertilized egg showing the vacuolate periplasm. × 2200.

Fig. *R*. A later stage in the development of the periplasm, showing the angular appearance of the vacuoles. Due to being mounted in glycerine, the contents of the egg have shrunk somewhat, revealing the thin periplasmic wall. × 2200.

Fig. *S*. A still later stage in the maturation of the periplasm. The intervacuolar material has coalesced, forming the characteristic reticulations. × 2200.

Fig. *T*. Optical section of the mature oöspore, showing the protoplasm surrounded by an innermost narrow endospore wall, a reticulate exospore wall and an outermost, thin periplasmic wall which encloses a gel-like substance. × 2200.

Fig. *U*. Surface view of the mature oöspore showing the characteristic reticulations. × 2200.

Fig. *V*. Optical section of a mature oöspore showing a persistent fertilization tube, which, in this case, was continuous with the exospore wall. × 2200.

A COMPARATIVE STUDY OF SCLEROTIUM ROLFSII AND SCLEROTIUM DELPHINII

F. L. Stevens

(With 16 Text Figures)

In July, 1929, specimens of diseased *Delphinium* were received from Normal, McLean County, Illinois, with the statement that in 1928 it appeared only on species of *Delphinium*, but in 1929 on "anything that grows in the infested regions, regal lilies, golden banded lily of Japan, false dragon head, *Phlox*, *Iris*." Later letters also reported it on a rag weed, butter and eggs and pink and blue spider wort, *Verbena*, Madonna lily, tulips, *Funkia* lilies, blackberry lily, *Pyrethrum* daisy, yellow daisy, violets.

In September, 1929, specimens of diseased *Delphinium* root rot were received from Williamsville, Sangamon County, Illinois, with the report that the disease had been troublesome for two years in spots and was getting worse. Carrots were reported to have the same disease, during dry weather, but not in wet weather. The cause of the disease in the two instances reported above was clearly *S. Delphinii* Welch, but the two strains differed slightly. This fact and the obvious relation of this fungus to *Sclerotium Rolfsii* led to a somewhat extended comparison of these two organisms, which I designate respectively as *S. Delphinii* I and *S. Delphinii* II, with *Sclerotium Rolfsii* and with two strains of *S. Delphinii*, one an authentic culture, No. S 637 from Dr. Whetzel of Cornell, hereinafter designated merely as *S. Delphinii*, the other a culture from the University of Wisconsin which I designate as *S. Delphinii* III.

The *S. Rolfsii* here used was of the same character so far as I can judge as that which I sent to Professor Saccardo and which thus came to be the type of the species. This also is the same in character as the one that was received by Dr. Halsted from Professor Rolfs, which I personally studied and which is figured in Plant Disease Fungi, p. 438.

Professor Trotter kindly loaned me the type specimen from Saccardo's herbarium, but it is now entirely devoid of sclerotia and was dead, so that no cultures were obtainable.

I present first a description of this strain of *S. Rolfsii* as it usually appears on rice or carrots which may I think under the circumstances properly be regarded as an amplification of the type description and which may help to clarify the status as regards the numerous races of this fungus that have been reported. Dr. Higgins says that he has fifteen isolations which

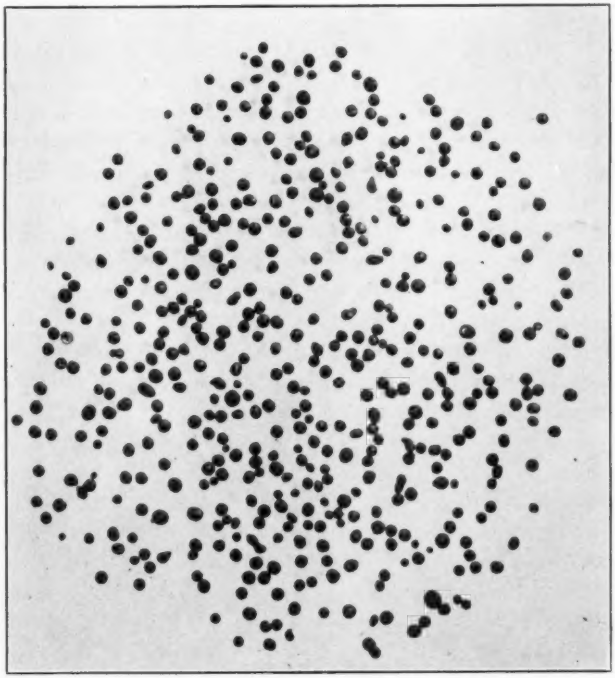

FIG. 1. Sclerotia of *S. Rolfsii* grown on carrot showing regularity in size and shape. See graph 1.

may probably represent five strains. Nakata [1] states that he has studied thirty-three strains. Edson and Shapovalov [2] report two strains.

[1] Nakata, K. Studies on *Sclerotium Rolfsii* Sacc. Part V. Physiological characters in relation to the strains of the fungus. Bull. Sc. Fac. Terkultara Kyushu Imp. Univ. **2**: 237. 1927.

[2] Edson, H. A. & Shapovalov, N. Parasitism of *Sclerotium Rolfsii*. Jour. Agr. Res. **23**: 41. 1923.

The sclerotia of *S. Rolfsii* are quite small, usually about 1.39 mm. in diameter (see also TABLE I), usually nearly or quite globose (FIG. 1), occasionally slightly irregular, and are very uniform in size. They arise in plexi formed in the aërial mycelium and with sufficient nutriment are very numerous, on corn-meal agar about 10 per square centimeter. They are usually borne singly but in rare instances two or more may coalesce. At first only a structureless white knot of tangled mycelium is seen without definite boundary or cortical differentiation. This soon assumes, one day, a globose form, definite boundary, and a cortical layer develops precisely as is the case with *S. Delphinii* described below. As these changes proceed the surface color changes first to pinkish buff, then to olive-brown and finally when old and dry to clove brown.[3] The surface is smooth, without markings, though liquid exudes as does from the sclerotia of *S. Delphinii*. The plexi which develop into sclerotia are borne on the aërial mycelium and the sclerotia are therefore usually free and suspended.

The sclerotia germinate by emitting numerous single mycelial threads between the cortical cells. The mycelium of the fungus consists either of long straight comparatively thick (7 mm.)

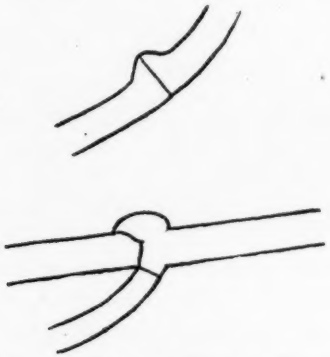

FIG. 2. *S. Rolfsii* showing clamp connections.

filaments, or much thinner and more crooked threads. The mycelium bears numerous typical clamp connections (FIG. 2).

[3] Colors from Ridgway standard.

The sclerotial plexi are composed of the more slender filaments. Always when sufficient humidity obtains there is a very dense development of aërial mycelium, floccose, wooly, often one to two centimeters in depth and so dense as to obscure vision of anything below it (FIG. 3).

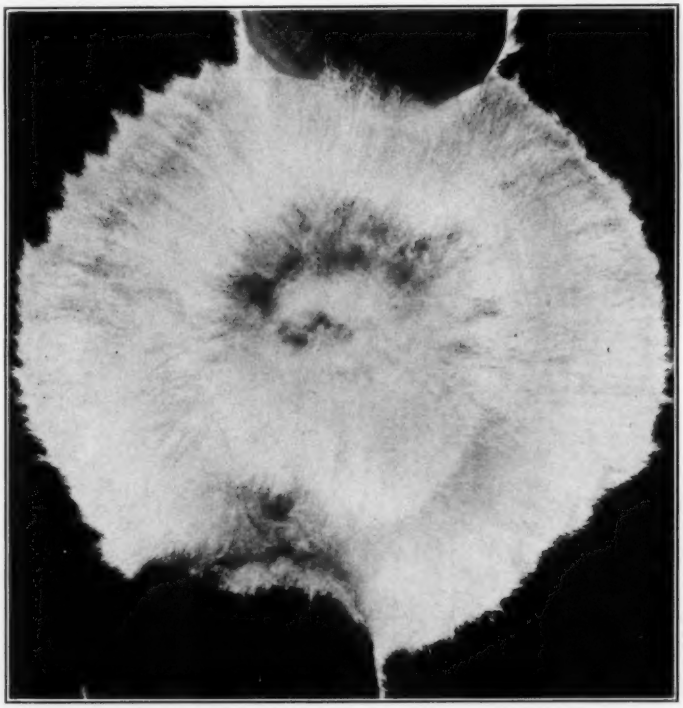

FIG. 3. *S. Rolfsii* growing on sterilized carrot, 5 days after inoculation, the aerial mycelium very floccose. × 1½.

When *S. Rolfsii* was grown on corn-meal agar and the Petri dish inverted, it gave much floccose aërial mycelium reaching down to the Petri cover and producing numerous sclerotia suspended in air between the agar and the dish cover. Exceptions to the usual appearance of *S. Rolfsii* as given above are as follows:

Sclerotium Rolfsii when grown on rather poor nutrients as on tap water agar therefore with more scant development of mycelium than otherwise gives rise to ropy mycelial columns just as is the case with *S. Delphinii* and on these columns the sclerotia are borne. *S. Rolfsii*, which is usually characterized by its small regular sclerotia, under some conditions forms much larger sclerotia and these much less regular in form. *S. Rolfsii* sometimes germinates by a mycelial fascicle just as does *S. Delphinii*, and this may even bear a new sclerotium on its tip. Though ropy mycelium was usually not developed by *S. Rolfsii* this character was well developed on potato dextrose agar.

The description of *S. Delphinii* taken from the type culture received from Dr. Whetzel as it usually appears when growing on rice follows:

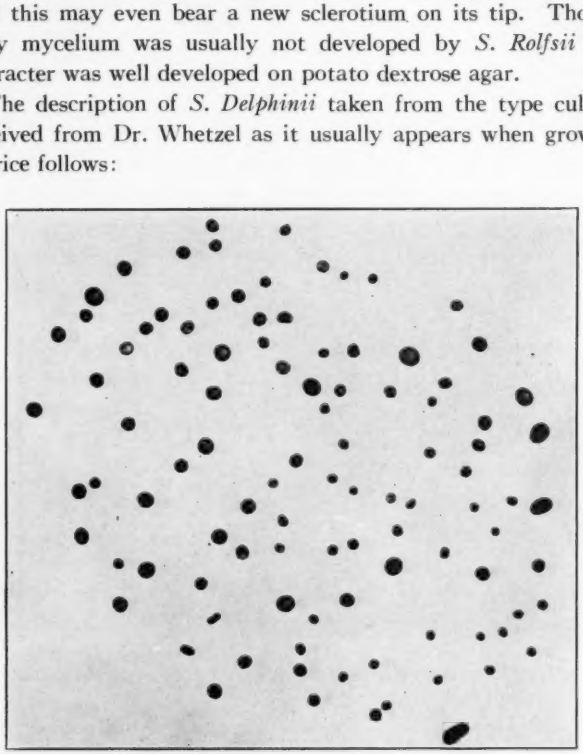

FIG. 4. Sclerotia of *S. Delphinii* as grown on corn-meal agar. See graph 2 for size.

The sclerotia vary greatly in size (FIG. 4), the mode being for the single sclerotia, 2.18 mm. in diameter, with a maximum of

5.18 mm. Irregular probably composite sclerotia may be 3.9 × 9.8 mm. across. The single sclerotia are sub-globose, arched on top, but are hollowed below so that when inverted they are saucer-shaped and bear in the center a hilum-like scar, the place of attachment to the stalk that bore them. They often coalesce in considerable numbers, thus forming a crust of very irregular shape and size. The sclerotia are borne on the apices of mycelial columns (FIGS. 5, 6, 7) which are from 0.5–3 mm. thick and 2–5 mm. tall, which arise from the substratum and which are composed of very numerous fascicled mycelial threads running parallel. The sclerotia first appear as a white globose enlarge-

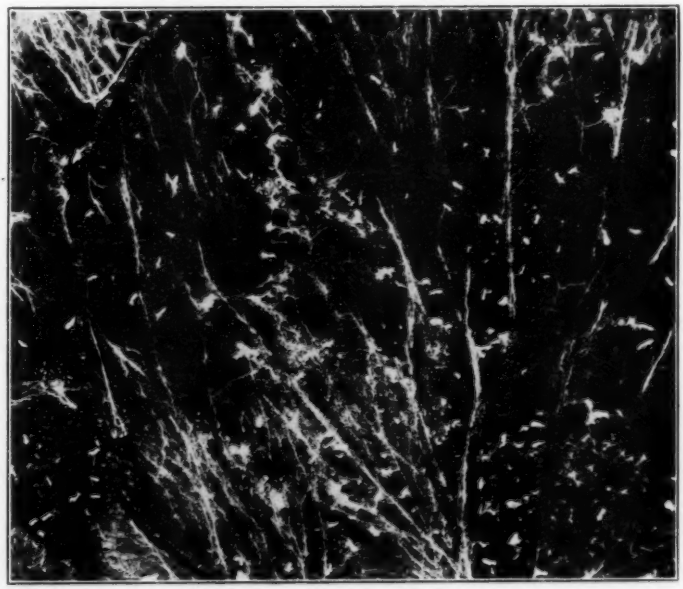

FIG. 5. *S. Delphinii* showing the sclerotia borne on stalks.

ment at the apex of the mycelial fascicle. Later they enlarge much in lateral diameter though but slightly in depth. At first they are without definite boundary or cortex, but they soon become of even surface and a cortex one cell thick develops. *S. Delphinii* killed six days after inoculation while the sclerotia

were still white showed the sclerotia globular and well delimited at the surface. A sclerotium 1.386 mm. in diameter possessed a very definite cortical layer about 30 μ thick. This cortex is composed of short, thick, irregular cells so intimately in contact with each other that no intercellular spaces appear. All of the region of the sclerotium within the cortex is uniform in character and consists of cells of very irregular shape, some long, some short and with many intercellular cavities. There is a larger proportion of long cells than is the case with *S. Rolfsii* (FIG. 8). If the sclerotia enlarge to over about 1.5 mm. in diameter they exude clear liquid at numerous points on the surface. The points of exudation appear different in color from the surrounding cortex. They vary in size from 540 μ in diameter downward to mere points and when large remain distinctly visible on old dried sclerotia (FIG. 9). Fresh sclerotia in section show the region immediately under these spots to have a water soaked appearance which extends downward into the medulla to a depth about equal to the diameter of the spot, but no morphological difference in them is discernible.

FIG. 6. *S. Delphinii* showing the stalks of the sclerotia.

The sclerotia as they age pass to ochraceous-buff, then to tawny and finally to Hay's brown.[4] The sclerotia usually germinate by emitting mycelial sheaves from the surface spots, these sheaves being very like those that bear sclerotia. Indeed, if conditions do not favor continued growth each sheaf may immediately terminate in a new sclerotium, so that in some instances a large sclerotium may bear twenty or more mycelial sheaves each with a new sclerotium at its tip. Such sclerotia are globose, smooth, unmarked and smaller than the usual sclerotium of *S. Rolfsii*.

The mycelial threads are quite like those of *S. Rolfsii* except

[4] Colors from Ridgway standard.

that the coarser threads are a trifle thinner, 5.4 μ, and in usual conditions they aggregate into ropy dendritic strands, Figure 10, which are composed of very numerous individual filaments. There is almost entire absence of aërial mycelium. When *S. Delphinii* was grown on corn-meal agar and the Petri dish inverted, it made no aërial mycelium or very little and that little was aggregated into mycelial ropes. The mycelium bears numerous typical clamp connections (FIG. 11). In this as in the other strains they are most easily demonstrated by growing the fungus on potato dextrose agar in inverted plates and examining the aërial mycelium.

Exceptions to the usual appearance of *S. Delphinii* as given above are as follows: The surface liquid exudation and attendant

FIG. 7. *S. Delphinii* showing a single sclerotium on its stalk.

surface marking so almost universal in this species are much less pronounced in some conditions and quite absent in others, for example, in a dry atmosphere or when sclerotia are so poorly nourished as to remain small. In the absence of suitable nutriment as on corn-meal agar and especially on tap water agar sclerotia remain very small (see TABLE I), uniform in size and regular.

There are several important characters that usually, at a glance, serve to separate *S. Rolfsii* from the four strains of *S. Delphinii*. First among these is the absence in *S. Rolfsii* of a stalk to the sclerotia. These, however, are not always absent and in some cases appear quite as typical as in the case of *S. Delphinii*.

Second is the surface marking of the sclerotium. This is never seen in *S. Rolfsii* and is usually present in *S. Delphinii*. However, if *S. Delphinii* is cultured in such poor nutriment as to

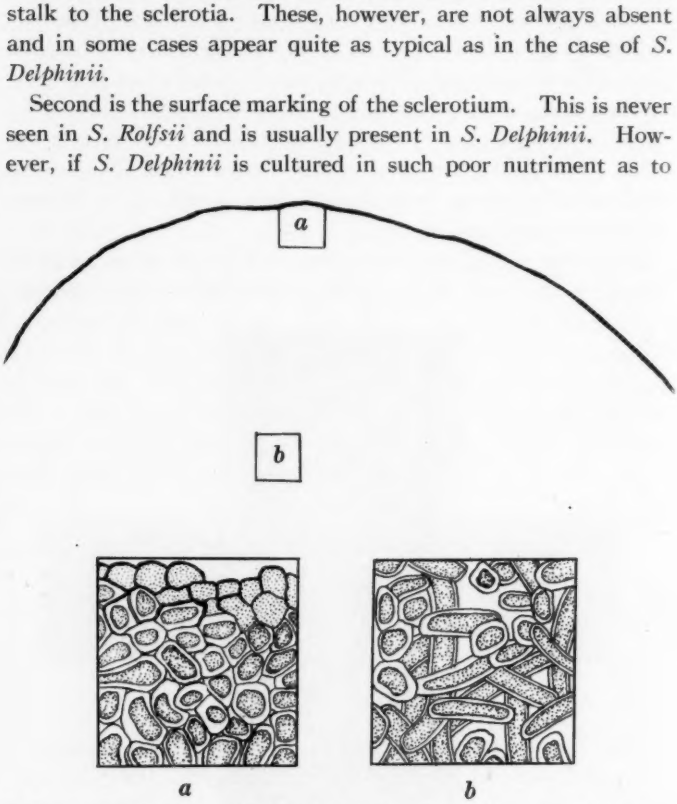

FIG. 8. *S. Delphinii*. Section of sclerotium; *a*, showing cortex, *b*, showing deeper region.

give only small sclerotia no pits develop. The small uniform sclerotia of *S. Rolfsii* contrast strongly with the large irregular sclerotia of *S. Delphinii*. Yet with absence of proper nutrients *S. Delphinii* forms sclerotia that are small and quite uniform in size, while on suitable nutrients *S. Rolfsii* forms sclerotia much larger, more irregular in size than usual, also more irregular in

shape. Germination of the *S. Delphinii* type of sclerotium is by the emergence of a dense fascicle of mycelium from one of the surface marks while *S. Rolfsii* usually germinates by single mycelial threads. In some cases *S. Rolfsii*, however, is seen to send forth a fascicle and this may even bear a new sclerotium on its tip.

S. Rolfsii usually shows an abundant floccose aërial mycelium, not ropy, while that of *S. Delphinii* is not floccose but is ropy. Under some conditions, however, *S. Rolfsii* assumes the ropy form though *S. Delphinii* never becomes floccose. The sclerotia of *S. Rolfsii* are never saucer shaped below, those of *S. Delphinii* are, but when poorly nourished *S. Delphinii* produces small sclerotia that do not show this character. *S. Rolfsii* makes very numerous sclerotia; *S. Delphinii* much fewer, but this difference is removed under certain conditions. *S. Rolfsii* and *S. Delphinii* so far as I have seen always differ in color of mature sclerotia as has been indicated above. Thus it is seen that while readily separable, if conditions are favorable for separation, these two forms lose nearly all of their differentiating characters under certain conditions.

Tabulated this appears as follows:

	S. Rolfsii	*S. Delphinii*
Mycelium	Densely floccose †	Not floccose *
	Not ropy †	Ropy *
Sclerotia	Not concave *	Concave †
	Not surface marked *	Surface marked †
	Color pinkish buff to olive brown to clove brown	Color ochraceous buff to tawny to Hay's brown
	Very numerous †	Less numerous †
	Small *	Larger †
Germination	Single threads †	Fascicled †

A * indicates that the character is constant; a † that it is not.

The only really differentiating character in all conditions is sclerotial color.

The four races of *S. Delphinii* are very closely alike though in certain conditions they show such differences that they must be regarded as four distinct strains. For example it is found that when inoculations of two of these races are made upon corn-

meal agar in one Petri dish the two colonies grow toward each other until they nearly meet. Then at the line of junction of the two a band, very distinct to the naked eye, about 3 mm. wide, and extending completely across the plate, is seen. Microscopically this line is seen to be caused by a very abnormal profuse irregular branching together with many smaller cells where the two colonies meet. Such a band was produced whenever any one of the five races considered above was grown on the same dish with any other of the five races.

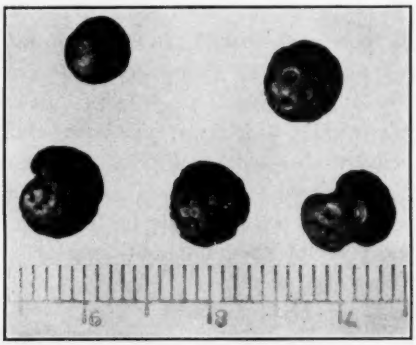

FIG. 9. Sclerotia of *S. Delphinii* enlarged mush, showing surface markings.

When any races were grown against another colony of the same race the mycelium of the two colonies mingled freely and no abnormal branching resulted. In none of the six oppositions was any consistent influence upon the number of sclerotia formed observed though occasionally sclerotia were more abundant where two colonies met, nor did most close search reveal any spore forms. Taubenhaus [5] stated that in his experiments sclerotia were more numerous where colonies met which he interprets as due to the existence of plus and minus strains. This appears to me extremely doubtful.

It seems probable though it is difficult to determine with certainty that when *S. Delphinii* II and *S. Rolfsii* are planted

[5] Taubenhaus, J. T. Recent studies on *Sclerotium Rolfsii* Sacc. Jour. Agr. Res. **18**: 127. 1919.

together the profuse branching is of the *S. Delphinii* II colony. When *S. Delphinii* I and *S. Rolfsii* come together the abnormal branching is of the *S. Rolfsii* colony.

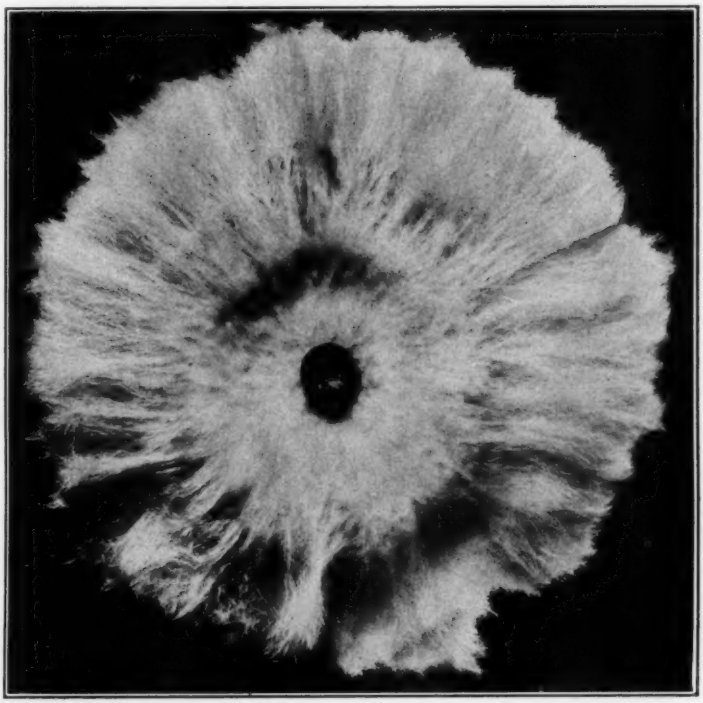

FIG. 10. *S. Delphinii* five days after inoculation, dendritic, not fluffy, ×1½.

It appears therefore that *S. Rolfsii* exerts a repellent antagonistic effect on *S. Delphinii* II and that *S. Delphinii* I exerts a similar effect upon *S. Rolfsii*. This antagonism is of the class discussed by Porter [6] and indicates here a difference between the strains showing it and does not appear when like strains were planted together. The five races were grown on various media

[6] Porter, C. L. Concerning the characters of certain fungi as exhibited by their growth in the presence of other fungi. Amer. Jour. Bot. **11**: 168. 1924.

TABLE I

SHOWING THE SIZE OF SCLEROTIA OF VARIOUS RACES ON VARIOUS MEDIA EXPRESSED IN MILLIMETERS OF DIAMETER

	S. Rolfsii	*S. Delphinii*	*S. Delphinii* III	*S. Delphinii* I	*S. Delphinii* II
On steamed rice					
mode		2.189	2.189	3.283	2.189
max.		5.174	4.975	6.567	6.567
min.		1.691	1.691	1.691	1.094
On second reading					3.223
					6.268
					1.592
On carrot					
mode	1.393				
max.	2.985 see Fig. 1				
min.	.950				
Tap water agar					
mode	.995	.895	.796	.895	.696
max.	1.194	1.293	2.089	1.393	1.791
min.	.574	.796	.597	.796	.696
Halved orange					
mode	1.791			3.183	3.183
max.	2.686			5.472	5.472
min.	1.393			2.189	1.890
On corn-meal agar					
mode		1.791	1.592		1.393
max.		3.383 see Fig. 4	2.487		2.487
min.		1.194	1.194		.796
Potato dextrose agar					
mode			2.189		
max.			4.975		
min.			1.891		
Mush					
mode	2.189			3.283	
max.	4.676			6.567	
min.	1.691			2.189	

and measurements of the sclerotia were made and are recorded below.

FIG. 11. *S. Delphinii* III clamp connections.

In addition to the sclerotia represented above there were in some cases more or less numerous, very irregular sclerotia, in some cases composites by coalescence of several, which could not with fairness be represented in the general tabulation. On rice in the case of *S. Delphinii* there were 16 such ranging from 2189 to 6069 wide by 3.880 to 9850 μ long.

S. Delphinii III gave 22 of these, width 2.189–5.372 × 4.378–8.756 μ.

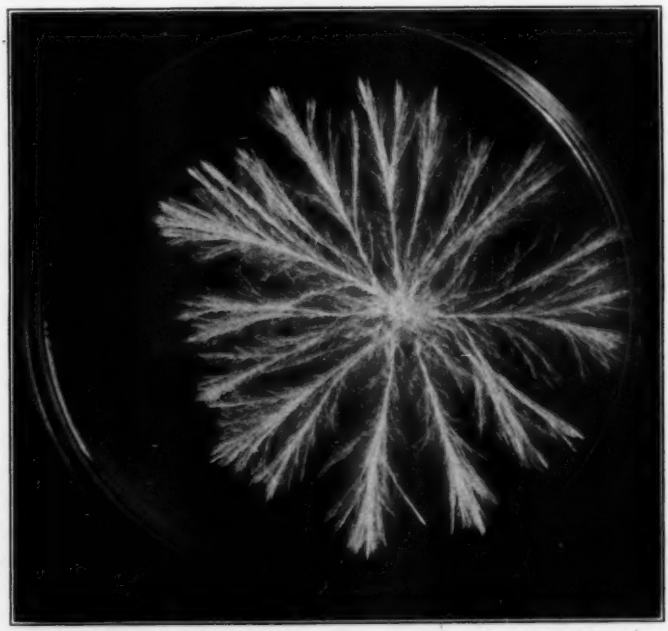

FIG. 12. *S. Delphinii* II on corn-meal agar showing dendritic growth.

S. Delphinii I gave 19 of these, width 2.189–5.870 × 5.372–9.850 μ.

S. Delphinii II gave 8 of these, width 2.786–5.372 × 5.372–9.850 μ.

On tap water agar, *i.e.*, without any added nutrients, growth was scanty with all five races and very few sclerotia were formed, more by *S. Rolfsii* than by any other race. The sclerotia in all cases were small. The modal differences in the tables are not significant because the sclerotia occurred in too small numbers, though it is significant that all sclerotia were very small, also that all were devoid of surface pitting. Graphs showing the sclerotial size as growing on rice are given in Figure 13. There is close agreement here between *S. Delphinii* and *S. Delphinii* III and *S. Delphinii* II but *S. Delphinii* I seems to differ.

S. Rolfsii was graphed only on carrot because it was usually so uniform in the size of its sclerotia.

An increase in size of sclerotia is shown by *S. Rolfsii* on corn mush over that on any other medium tried. On orange there was slight increase in the mode over that on carrot. Cornmeal agar shows its inferior nutrient value with the races of *S. Delphinii* in that the sclerotia are distinctly smaller than when these races are grown on rice. Potato dextrose agar, however, was equal to rice in value for *S. Delphinii* III as was also mush for *S. Delphinii* I. The carbohydrate here is probably the needed food.

All five races were grown on potato dextrose agar and irradiated when the colonies were 6 cm. in diameter with ultra-violet for various dosages. All showed a distinct stunting effect with 30 seconds' irradiation, and some stunting with five seconds, and sclerotial formation was permanently inhibited on the irradiated area (FIG. 14), though they did form on the new growth arising from the irradiated area. One day after irradiation a distinct band of aërial mycelium about 4 mm. wide was visible at the locus of the irradiated mycelial tips in *S. Delphinii* II and *S. Delphinii* I.

Dr. Nakata very kindly sent me six strains of *S. Rolfsii* studied by him, namely No. 501 isolated from potato at Kyushi, Uni-

versity Agriculture Farm, No. 502, from petunia at Kyushi, No. 519, from carrot at Kyushi, No. 524, from Konjac (*Amorphophallus*) at Kyushi, No. 527, from sugar beet in Korea, No. 509, from Konjac at Takushima.

While I am unable to make extensive comparisons of these with my strains the following notes may be worth publishing:

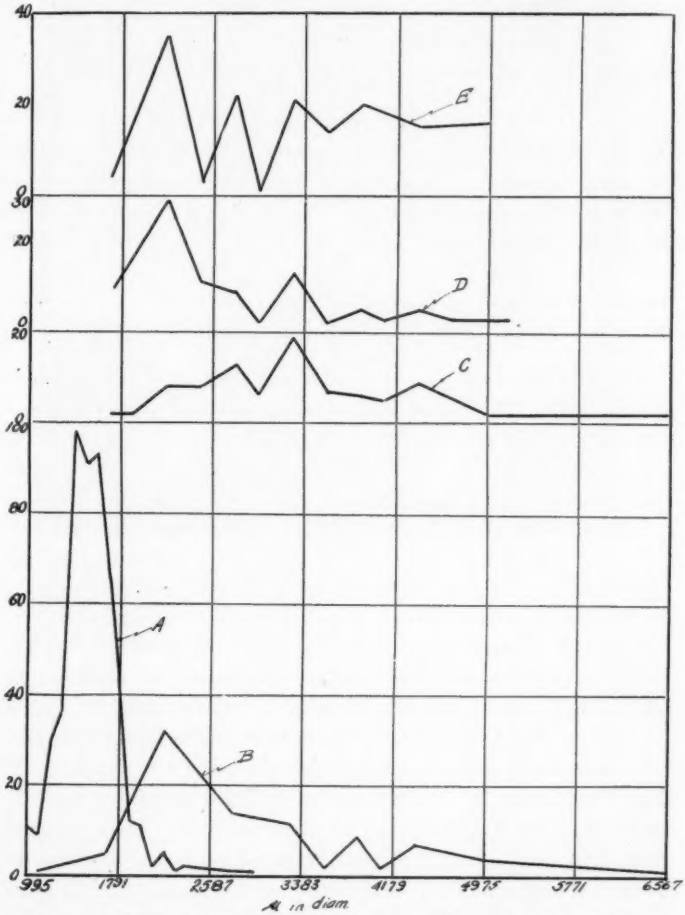

FIG. 13. Graphs showing sclerotial size of five strains of *Sclerotium*. A, *S. Rolfsii*; B, *S. Delphinii* II; C, *S. Delphinii* I; D, *S. Delphinii*; E, *S. Delphinii* III.

ON CORN-MEAL AGAR

No. 501. Very like *S. Rolfsii*, sclerotia a trifle larger.
No. 502. As above.
No. 519. Sclerotia a trifle smaller than above numbers and frequently showing stipes.
No. 524. As in No. 501.
No. 527. Sclerotia twice the diameter of those of No. 501 and few and showing surface spots, stipes common.
No. 569. As in No. 501.

ON POTATO DEXTROSE AGAR

No. 501. Much as on corn-meal agar but growth more luxuriant.
No. 502. As above, but of very different growth habit.
No. 519. Sclerotia very few.
No. 524. Sclerotia very few, but habit different from that of No. 519.
No. 527. Growth habit very different from all others.
No. 569. Much like No. 501 but with much fewer sclerotia.

Comparison of the results on these two media gives conclusive evidence that these six strains show considerable differences and since in none of these was there the abundant aërial mycelium so characteristic of my *S. Rolfsii;* they also differ from the five cultures that I am studying.

To ascertain the relative rapidity of migration through soils of various degrees of wetness glass tubes 13 mm. inside diameter were cut 5 cm. long and fitted at each end with corks each bearing four longitudinal grooves to allow exchange of air and moisture. These tubes were filled with soil and water added 1, 2, 3 and 0 cc. per tube. Three cubic centimeters of water made the soil very wet. Each tube was inoculated with *S. Rolfsii* Jan. 20 by placing a mass of mycelium under the cork at one end. The tubes were then placed on glass benches in a moist chamber. The photograph Fig. 15 was taken Jan. 24, showing growth in direct relation to the amount of water present. At seven days from inoculation the soil that received no water showed no growth of the mycelium; that with 1 cc. and 2 cc. had advanced 17 mm. each while that with 3 cc. had advanced 4 cm.

To determine the rate of growth in soils of different qualities

tubes like those described above were used with each of the following: rich loam, horse manure, sand, soil and manure and soil and sand, and tests made of rate of lineal growth through the

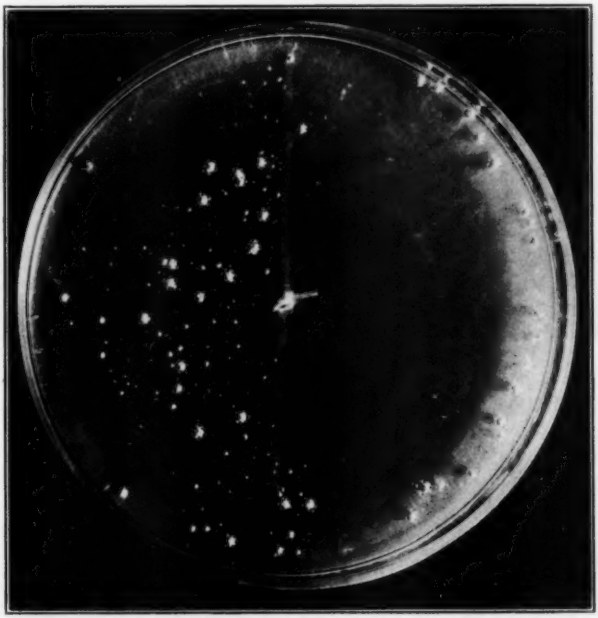

FIG. 14. *Sclerotium Delphinii* showing complete suppression of sclerotia by 30″ ultra-violet irradiation.

tube. Four tubes of each composition were inoculated separately with each of the five strains and half of them kept in the dark, the others in the light. The results are given in the following table with the average lineal growth expressed in millimeters.

TABLE II

	S. Delphinii	*S. Delphinii* III	*S. Delphinii* I	*S. Delphinii* II	*S. Rolfsii*
1. Loam.....	45 mm.	45	45	45	45
2. Manure.....	24 mm.	12	22	12	15
3. Sand.....	25 mm.	25	23	12	45
4. Soil and manure.....	45 mm.	45	45	45	45
5. Soil and sand	12 mm.	17	30	8	45

It is seen that growth is most rapid in loam or loam and manure and that it is about equal for the five races. The growth in manure was very poor as was also the growth in sand or sand and loam, with the exception however that *S. Rolfsii* grew at its maximum in each tube except that containing the manure.

FIG. 15. Showing relative rate of growth of *S. Rolfsii* in soils of different water content; from left to right was added 0, 1, 2, 3 cc. of water.

The experiment was repeated using each of the soils mentioned above but employing two series of soils, one loosely filled in, the other tamped in. No difference whatever was noticeable between the growth in the tamped and the loose soil.

King and Loomis,[7] who made somewhat similar experiments, state that "After trial of various types of soil, it was found that the mycelial strands grow most rapidly in pure sand."

From all of the facts it appears that all of the races here considered are very closely related and might indeed be considered merely as varieties of one species, though the considerable differences presented between *S. Rolfsii* and *S. Delphinii* may warrant the retention of the latter as a separate species.

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[7] King, C. J., and Loomis, H. F. Further studies of cotton root rot in Arizona with a description of a sclerotium stage of the fungus. Jour. Agr. Res. **39**: 665. 1929.

GLOEOSPORIUM ROSAE, A NOMEN NUDUM

ANNA E. JENKINS

Gloeosporium Rosae Hals. (**3**) causing a destructive rose (*Rosa*) disease, termed anthracnose (**3**), is apparently to be regarded as a *nomen nudum*. The disease was considered by Halsted as possibly identical with anthracnose of brambles (*Rubus*). As discussed by him, however, practically all of its etiological and symptomatological features (**3**, **4**, **5**) are unlike those of the latter distinctive disease. In the main they suggest a disease more like those caused by Sphaeriales, such as *Glomerella* or *Diaporthe*. Insufficient data were given by Halsted even to establish the generic identity of the organism to which the name *Gloeosporium Rosae* was applied. Through correspondence with Elizabeth Clark, New Jersey Experiment Station, it has been learned that the specimens studied by Halsted are not available there. None were found by the writer in the Mycological Herbarium of The New York Botanical Garden and in that at the Bureau of Plant Industry. Through Dr. W. H. Weston it has been learned that such specimens are not to be found in the Farlow Herbarium at Harvard University. Thus no basis has been found on which to establish the identity of the fungus associated with Halsted's rose anthracnose. The binomial *Gloeosporium Rosae* is not mentioned in such standard mycological works as Saccardo's Sylloge Fungorum. It is found in a number of references pathological in character. In some of them its mention is based directly upon data presented by Halsted, although in one instance (**1**: 92) it represents material of which a part was recently recognized by the writer as *Diaporthe umbrina* Jenkins. Seymour's (**9**: 396) listing of *Glomerella cincta* (Stoneman) Spaulding and von Schrenk as the perfect stage of *Gloeosporium Rosae* is based upon publication to this effect by Schwartze (**8**: 58).[1]

Reference (**2**) to anthracnose "affecting the raspberry, blackberry and rose" probably has a direct relation to Halsted's

[1] Information from the files of A. B. Seymour, contributed by C. W. Dodge.

suggestion that the anthracnose of brambles and his rose anthracnose may be identical diseases. Since it is not known to what rose disease Halsted applied the term anthracnose, that name as so employed by him is now without significance. It has been explained previously (**6**) that Sheldon (**10**) may have discussed under the title of "Some rose anthracnoses" the rose disease now known as brown canker. That other rose diseases which he (**10**) referred to at the same time are not definitely diagnosed has been learned through correspondence or personal conference with Professor Sheldon.

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3. **Halsted, B. D.** Fungous troubles of the rose. New Jersey Agr. Exp. Sta. Rept. **13** (1892): 280–283. 1893.
4. ——. The anthracnose of the rose. New Jersey Agr. Exp. Sta. Rept. **14** (1893): 401–405. 1894. (Illus.)
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10. **Sheldon, J. L.** Some rose anthracnoses. Phytopathology **3**: 70. 1913.

AMANITA CALYPTRATA AND AMANITA CALYPTRODERMA

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In January, 1900, Peck [1] described *Amanita calyptrata* Peck from Oregon material sent in by Dr. H. Lane during the autumn. Another Pacific Coast species closely related to the latter was described in 1909. It is very interesting but not strange that two great American mycologists, George F. Atkinson and Charles H. Peck, should within six days publish descriptions of and give similar names to this *Amanita*. Both received material from California. Atkinson received material during the fall and winter of 1908 from Mrs. V. G. Ballen, of Brookdale, Santa Cruz County. Mrs. Ballen provided such adequate, distinguishing notes, photographs and specimens that it was published jointly as *Amanita calyptroderma* Atkinson & Ballen. This description was published first in Science, June 11, 1909.[2] Only six days later was issued Peck's description of the same plant under the name *Amanita calyptratoides* Peck.[3] Peck's name refers to the similarity of this plant to his previously described *A. calyptrata*, while Atkinson and Ballen use the name *A. calyptroderma* "because the calyptra of the volva fits like a skin over the center of the pileus."

A description of the local distribution in California, ecology and field notes on *A. calyptroderma* appeared later the same year.[4] Herein is mentioned the close relationship of this species with the more robust European *A. caesarea* and Peck's *A. calyptrata*.

Murrill [5] and Kauffman [6] have given preference to Peck's

[1] Peck, Chas. H. New species of fungi. Bull. Torrey Club **27**: 14. 1900.

[2] Atkinson, G. F. A new edible species of Amanita. Science, N. S. **29**: 944. June 11, 1909.

[3] Peck, C. H. New species of fungi. Bull. Torrey Club **36**: 329–330. June 17, 1909.

[4] Atkinson, G. F. A remarkable *Amanita*. Bot. Gaz. **48**: 283–293. *Illus.* Oct. 1909.

[5] Murrill, W. A. Agaricaceae. *Venenarius*. N. Am. Fl. **10**: 71; 75. 1914.

[6] Kauffman, C. H. Fungous flora of the Siskiyou Mountains in southern Oregon. Mich. Acad. Sci., Arts, and Letters, Papers **11**: 180. 1930.

name because they evidently overlooked Atkinson's description in Science, but they have referred doubtless to a description published later by Atkinson.[7] Murrill gives to *A. calyptrata* Peck the new name *Venenarius Lanei* because of the pre-Friesian *Amanita calyptrata* Lam. 1778. Kauffman evidently had not seen Atkinson's [4] discussion and description of the double cup at the base of *A. calyptroderma*.

These two species, *A. calyptrata* Peck and *A. calyptroderma* Atkinson and Ballen, are very similar and difficult to distinguish. The main distinction is the greenish tinting of the pileus and gills in *A. calyptrata*, and the thick double volvate cup at the base of the stem in *A. calyptroderma*. Fortunately, both species are edible. The Italians consume many of them and very likely presume them to be identical with their old acquaintance, *A. Caesarea*, of their native land.

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[7] Atkinson, Geo. F. Preliminary notes on some new species of Agaricaceae and *Clavaria*. Ann. Myc. **7**: 365. August, 1909.

HERBARIUM ARRANGEMENT OF MYCOLOGICAL SPECIMENS [1, 2]

E. T. Bartholomew

(With Plates 21 and 22 and 4 Text Figures)

How shall the specimens be arranged so that they will be most readily accessible and at the same time be best preserved, is one of the questions that presents itself to every herbarium curator who wishes to build up an efficient mycological herbarium. The same question also arises when it becomes advisable to rearrange the specimens in an already existing herbarium.

A mycological herbarium to be of the greatest value to those who wish to use it should have its specimens so cared for that they will continue to remain as nearly as possible in the original state of preservation, and it must be workable. It should have the information which it contains as logically arranged and as readily available as the information in a dictionary or an encyclopedia. Probably this is an ideal which has never been actually attained, but more nearly so in some herbaria than in others. However, it has been the personal observation of the writer, and his observation has been corroborated through correspondence, that many, if not most, of the mycological herbaria in the United States are very much below par in their efficiency.

So far as the writer has been able to ascertain, the American literature contains no detailed discussions of this subject. It is for this reason that the methods of arrangement in use in some of the larger mycological herbaria in the United States are being described and discussed in this paper.

[1] Paper No. 224, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] It is with pleasure that the writer acknowledges the receipt of data and samples from numerous herbarium curators, and especially the helpful criticisms by Dr. H. M. Fitzpatrick of Cornell University, Dr. H. S. Jackson of the University of Toronto, and Dr. H. D. House of the New York State Museum, who read the manuscript.

METHODS OF FILING MYCOLOGICAL SPECIMENS

1. *Specimens in Packets Attached to Herbarium Sheets*

Flat, thin mycological specimens, for example, such as are found in the Phycomycetes, Ascomycetes and Basidiomycetes, are placed in specially-folded envelopes called packets.[4] In the majority of the herbaria these packets are attached to sheets of heavy, white paper, approximately 11 × 17 inches in size. This

No.	Name of Herbarium
	.....
S.Pkt. L.Pkt. Liquid Box Ill. Culture Slide Paraf. Neg. Photo. Lant.Sl. Letter Notes Dupl.	Hab..... Loc..... Leg.....Date..... Source.....Det..... Notes

FIG. 1. These or similar data will be found helpful if printed on the card that is to be used in the accession or species card-index file of the herbarium.

is the standard herbarium sheet used in mounting phanerogamic specimens. However, in some herbaria a sheet only half that size, $8\frac{1}{2} \times 11$ inches, is used. The packets are either glued or pinned to these sheets. If the latter method has been used the packets may be transferred to a different sheet with less danger of marring and making unsightly the one to which it was originally attached. Such transfers are desirable when, for example, it has been decided to change to a different system of classification. The size of the packet varies, but for the sake of conservation of space it conforms as nearly as possible to the size of the specimen which it contains.

[4] Perhaps it would be more correct to use the word "pocket" or "envelope" when referring to the folded receptacle alone and the word "packet" when referring to the folded receptacle containing a specimen, but for the sake of uniformity and to follow common usage only the latter will be used.

The large sheets, each bearing one or more packets, are put into folders of heavy manila paper and laid flat, one on top of another, in the herbarium pigeon hole or compartment. A separate folder may be used for each genus, for each species or for each species on a given host. The smaller-sized sheets may be filed in the same manner or, as in some herbaria, they may be placed in folders of proper size and filed vertically in letter-file fashion.

In some herbaria it is desired to indicate geographical distribution from different parts of the world without resorting to a complete segregation. This is done by having a different colored folder for different countries or groups of countries. For example, for collections from North America the folders may be the common manila, from Europe they may be blue, and from the Asiatic regions white. Folders for the collections from the local state may be of still another color. While such a plan would require more space than if all species of a given genus were filed regardless of distribution, it would probably require less space than if the different collections were segregated and kept in separate parts of the herbarium.

The following are a few of the herbaria in which all but the bulky specimens are placed in packets and attached to herbarium sheets: United States Herbarium in Washington, the Farlow Herbarium at Harvard, the Arthur Herbarium at Purdue, the Atkinson Herbarium at Cornell, The New York Botanical Garden Herbarium and the Missouri Botanical Garden Herbarium. In all of these the herbarium sheets are of standard size and are filed flat. At the University of Wisconsin, Iowa State Agricultural College, Brooklyn Botanic Garden, and in some private herbaria the packets are attached to sheets that are only one-half the standard size. In the two former they are filed flat and in the latter usually vertically.

ADVANTAGES OF METHOD.—By filing in this manner, all (but not an individual) of the species of a given genus or of a given host may be available with a minimum of time and labor; an entire infected host plant may be preserved; drawings or photographs of the fungus may be attached to the sheet beside the packet; the letter or separate containing the original description

of the species may be attached to the sheet which bears it; and the specimens lie flat and do not drop to the bottom of the packet, unless the sheets are filed vertically, as they are in a few instances where the sheets are only half size. For an example of how the packets, drawings and notes may be placed side by side on the same herbarium sheet see plate 21.[5] Perhaps one of the greatest advantages of this system lies in the fact that where the specimens are filed flat, herbarium cases of the same size and design may be used for all specimens, no matter what their size. Practically no specimens need to be reduced in size. The large ones, such as appear in the Basidiomycetes and Gastromycetes, may be placed in boxes and filed in the same compartment as the folder which contains the flat specimens of the same genus or species. This method also conserves the time of the curator in that usually the original packet and label are retained and all that is necessary for filing is to pin or glue the packet to the proper sheet.

DISADVANTAGES.—In order to examine an individual specimen the folder must be taken from the cabinet compartment, a suitable table space on which to open the folder must be located, and then several sheets may have to be shifted in order to find the desired specimen. In the hands of an expert the packet sheets can be handled or shifted without harm but a careless worker or the person who has had a minimum of experience in herbarium work may do much injury to fragile specimens by permitting the large sheets to bend excessively while he is handling them. This excessive bending may also release the fold at the end of the packet. Time must be taken to rearrange the fold or the packet sheet looks untidy; furthermore, if this is not done, portions of the specimen may protrude or even be lost from the packet. A comparatively large space is required in which to file specimens in this manner. Within the herbarium there may be a large number of the sheets that temporarily bear only one packet, thus requiring not only excessive filing space, but filing material. If the packet sheets are only half size and are filed vertically in the drawers of a letter file a stack of four drawers

[5] The photograph for this plate was kindly sent to me by Dr. J. C. Arthur of Purdue University.

is the most that can be used without requiring the use of a step ladder. Such an arrangement is not conservative of floor space. This plan is also subject to the objection that the specimen drops to the bottom of the packet and that there is more danger of crowding and breakage than if the sheets are laid horizontally. Specimens might be injured by accidentally inserting one folder inside of another when replacing a folder in the drawer. This is an objection which has been given, but it does not seem probable that it is an important one. Where packets of varying sizes are used a certain amount of space on the packet sheet cannot be utilized, and this is especially true when the half-size sheet is used.

2. *Specimens Filed Vertically in Packets or Envelopes*

In some herbaria the packets are not attached to herbarium sheets, but are filed vertically in boxes or drawers, like cards in a library card index system, with appropriate guide cards. In some cases the packets are filed just as they are, while in other cases each packet is first placed in an envelope and then filed. In the former, packets of a uniform size are used; in the latter they may be of a uniform size or there may be a certain amount of variation in the size of the packet, but the envelopes are all of a standard size. The size of the packet or envelope may vary with the herbarium, or more than one size may be used in the same herbarium.

In some herbaria all packets are put into a standard $3\frac{1}{2} \times 6$ inch envelope with a non-gummed flap. These envelopes are made of about twenty-pound manila paper, or of paper of similar weight and quality but of some other color. The latter are used where it is desired to designate specimens from a given locality without having to resort to segregation.

Where the packets are filed without the use of an envelope the same results may be obtained by using paper of different colors from which to make the packets. A similar result is also obtained in some herbaria where ink of different colors is used in typing or writing the data on the outside of the packet or envelope.

In at least one herbarium the packets are all uniform in size

(5 × 6½ inches) and then these are placed in a 5½ × 7¼ modified photographer's envelope. These envelopes contain the usual thumb and finger "cut-out" at the open end but the legend is written parallel with the long rather than with the short edge and the envelopes are filed accordingly.

In different herbaria where this system of filing is followed the packet or envelope appears to vary in size from 3 × 5 to 5½ × 7¼ inches. The former size is more likely to necessitate a cutting of the specimen but is more conservative of filing space than the latter size.

In the herbaria where the packets are pinned or pasted to sheets of heavy paper, boxes of various sizes are usually used in which to store the large, bulky specimens. The vertical filing system is also adapted to the filing of most of the larger specimens in this manner without their having to be cut. The box has a cover and its size is the same as that of the packet or envelope, except in thickness, and this is governed by the thickness of the specimen to be filed. A specimen in such a box may be filed in its proper order among the packets, thus avoiding segregation.

Where the packet is filed vertically without placing it in an envelope, the label is removed from the original packet and pasted to the standard packet, or a new label is made and the old one enclosed. In some cases, instead of making a new label, the data are typed or written on the upper fold or on the main body of the packet. If typed, the typing is done before the sheet of paper is folded into packet form. Where the packets are put into envelopes the original packet and its label are kept intact, or, if a new packet is made, the label is removed from the old packet and attached to or placed within the new one. If the latter plan is followed the data from the original label are written or typed on the outside of the envelope. In case the specimen is large enough to require a box which is to be filed vertically, either the original label or a new one is pasted on the outside of the box. If a new label is made the old one is placed in the box with the specimen.

The vertical system of filing specimens is especially well adapted to cross reference by means of a card index. The speci-

mens may be filed and indexed according to sequence of accession, according to host or according to order, genera and species. If the specimens are filed according to order of accession at least two card indexes are used, one listing the accessions serially and the other arranged according to host. A third index arranged according to genera and species is sometimes used. If they are filed according to order of accession a card similar to the one shown in figure 1 is used, and is usually filled out in writing. The data for the other one or two index files are obtained from this card and are usually typed. If the specimens are filed according to genera and species then only one card index system is necessary, namely, the host index, but in some cases a card index to fungus genera and species is also kept.

The use of the accession card as shown in figure 1 is probably self-explanatory. Heading the column on the left is the serial accession number. The genus and species of the fungus and the name of the author are placed on the top line. The remainder of the data are filled in as indicated. By checking or underscoring one or more words in the left margin one may indicate location, method or methods of preservation, etc., of a given specimen. The particular marginal words shown in figure 1 may be changed or substitutions may be made in order to fit them to the needs of any given herbarium. If the arrangement in the herbarium is such that an accession card is not used then a similar marginal checking system may be used to advantage on the host or genera and species index cards. It is perhaps unnecessary to figure or enumerate the other data that should be placed on these cards, except to say that on the host index card sufficient space should be left for the recording of the herbarium numbers of a given parasite, especially if the specimens are arranged according to host.

In the herbarium of the New York State Museum such specimens as those of the Thelephoraceae, *Peridermium* and the lower fungi are placed in packets and the individual packets are filed vertically. At Cornell the Plant Pathology Herbarium and the specimens in Durand's collection are filed in the same manner. The same system is used also in the Bartholomew Herbarium at Hays, Kansas, at the University of Missouri,

Massachusetts Agricultural College, Oregon Agricultural College, and in the Plant Pathology Herbarium of Washington State College. In some of these herbaria all specimens have been reduced, where reduction was necessary, to such a size that they could be placed in standard-size paper packets or in cardboard boxes which are filed vertically in their proper places among the packets.

ADVANTAGES OF METHOD.—It is conservative of both floor and filing space and a maximum number of specimens are available without the use of a step-ladder. Perhaps as many specimens per cubic foot of space could be filed in the herbarium where the packets are attached to large sheets and filed flat as where the packets are filed vertically, provided no vacant spaces were left on the large sheets in each filing compartment, but this is seldom if ever the case. As mentioned above this method is well adapted to the use of a card index system for cross reference. The easy accessibility of any single individual specimen is another favorable feature, except where the specimens have been filed serially in order of accession. By this method the specimens can be more easily rearranged according to a different classification, if such a change should appear to be desirable. Drawings, lantern slides, photographs and permanent microscopic mounts on glass slides may be filed in or adjacent to the packet or envelope containing the specimen. Other features which make this method of filing desirable are that it is necessary to handle only one specimen at a time, danger of breakage through handling is reduced to a minimum, the specimens are probably less accessible to insects and are as nearly as possible free from dust and smoke fumes, especially if the packets are placed in envelopes.

DISADVANTAGES.—Probably the greatest objection to this method of filing is that it may require the cutting of either a host or a fungus in order to get it into the packet, envelope or box. However, if the packets are at least 4 × 6 inches in size and if the boxes of similar size are used in conjunction with these, most of the specimens may be filed without having to resort to an objectionable amount of reduction. Where specimens are filed in this manner a mycological systematist would probably find it more cumbersome than by the method described

under the first heading. This would be especially true if each packet were filed in an envelope. For example, if several specimens of a given species were to be examined at the same time each packet would have to be removed from its envelope before the packet could be opened, while if the packets were attached to herbarium sheets the opening of the folder would at once expose all of the packets containing the specimens of a given species. While the filing of specimens in the order of accession is conservative of space and may have other advantages, yet such a system would be very inefficient for the use of a mycological systematist. One herbarium drawer might not only contain many species but also many genera; specimen number 500 might be an *Aecidium* and 501 a *Sphaerotheca*. If the packets are not placed in envelopes there is some danger that the end flaps may become doubled-over at the corners or may make more or less trouble in filing. By filing the packets vertically the specimens not only tend to shift to the bottom of the packet, but there is also danger that by overcrowding some of the more fragile specimens may be injured. Furthermore, this method, though using a minimum amount of floor and filing space, calls for a greater expenditure of time and labor, and for actual filing material such as paper for the making of standard-size packets for envelopes, for guide cards, and for the cards to be used for indexing.

3. *Specimen Packets on Sheets Bound in Book Form*

Comparatively few mycological herbaria in the United States have specimens filed in book form. Harvard, the University of Illinois, and the Connecticut Agricultural College may be cited as having at least a portion of their mycological specimens filed in this manner. In most herbaria the packets are removed from such volumes and filed in the same manner as the other specimens. This method, however, has the advantage that collections of exsiccati, for example, may thus be kept intact, preserving the individuality of the persons issuing them and also the nomenclature of the periods in which they were issued.

This method has the disadvantage that it is expensive, a special type of filing case is required, and the specimens of a given

species cannot be easily segregated. The last objection may be partially overcome or compensated for by using a loose-leaf volume, or by numbering the pages and making a card index reference list of the specimens.

4. *Specimens Filed in Boxes*

Large, bulky specimens, such as certain of the Basidiomycetes, are not usually filed in packets but in cardboard boxes of suitable size. The very small, fragile specimens of this same group or of other groups are sometimes filed in very small boxes and these

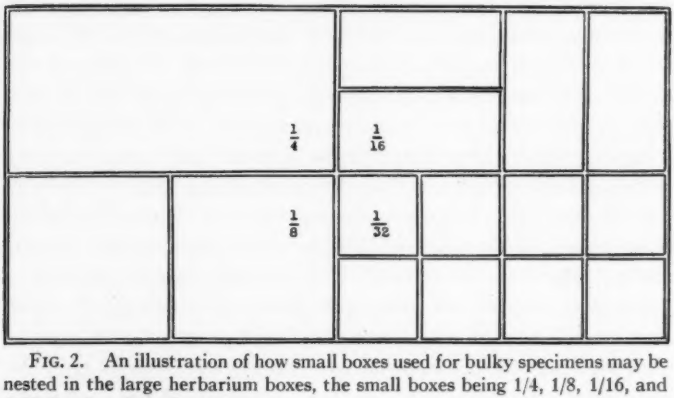

FIG. 2. An illustration of how small boxes used for bulky specimens may be nested in the large herbarium boxes, the small boxes being 1/4, 1/8, 1/16, and 1/32 the size of the large ones.

in turn are glued to the inside of the packet. To keep these very small specimens from being injured by rattling around in the box one or more of the following methods may be used. The interior of the box may be padded with cotton, or some prefer very soft cloth or tissue paper, thus avoiding any annoyance from loose cotton fibers; the specimen may be glued to the interior of either box or lid, the latter permitting more satisfactory inspection; it may be pinned to either box or lid by using short pins on the inside or longer ones which enter from the outside; to facilitate ease of specimen inspection it may be pinned or glued to a card which fits snugly into the box but which is not permanently attached to it; a slim-pointed thumb tack with its head glued to the bottom of the box may be used upon which to impale certain kinds of these specimens.

In a herbarium where the packets are attached to sheets, two groups of boxes (usually cardboard) are generally used in which to file the bulky specimens. The first group is composed of small boxes of various size, into which the specimens are placed, and the second group is composed of large boxes which serve as containers for the small ones. The large boxes have covers; the smaller ones may have covers, but usually do not. The lateral dimensions of the large box are usually such that it will just fit into the regular filing compartment, while its depth is usually such that from one to three may be filed, one on top of another, in the same compartment. For example, if the filing compartment is 5 inches high, one may be approximately $2\frac{3}{8}$ inches deep and the other two approximately $1\frac{1}{4}$ inches deep. The small boxes vary in size but are such that they can be nested within the large boxes without loss of space. Figure 2 illustrates how different combinations of the smaller boxes may be filed within the larger ones. Within the large box any two of the smaller boxes may be replaced by the one that is twice as large, or conversely, the larger sizes may be replaced by two that are just half as large, four that are one-fourth as large, etc. For the sake of neatness, the small boxes in a given large box should be of the same depth, and that depth should be just a little less than that of the large box in which they are filed. However, in some herbaria which have been examined the small boxes vary in depth according to the size of the specimen which they contain. Some herbaria also use only two sizes of small boxes, 2×3 and 4×6 inches, with depths ranging from $\frac{1}{2}$ inch to 2 inches. In another herbarium the small boxes (covered) are of six different sizes, ranging from $3 \times 2 \times \frac{6}{8}$ to $6 \times 8 \times 2\frac{1}{2}$ inches. These are placed in large covered cardboard boxes, approximately $16\frac{1}{2} \times 18\frac{1}{2} \times 4$ inches, two of which are filed side by side in a herbarium compartment.

In at least one herbarium the Clavariaceae and most of the resupinate Thelephoraceae and Hydnaceae are not put into the small boxes, but are put into packets ($5\frac{1}{2} \times 3\frac{5}{8}$ inches) made of stiff paper and filed vertically in the large boxes along with the smaller boxes, which preserves the natural order, or in covered boxes of full length and height, but only $5\frac{3}{4}$ inches wide.

In some cases covered cardboard boxes of suitable size are used for filing the forms that cannot be readily placed in the packets, but instead of nesting these small boxes within larger ones they are glued to stiff cardboards of the same size as the regular herbarium sheet. These are then pigeon-holed in proper order along with the genus and species folders which contain the flat specimens.

One herbarium, economizing on box expense, does not use the large box of herbarium-sheet size, but uses shoe boxes into which smaller boxes are nested. These boxes are about 6×12 to $12\frac{1}{2} \times 3\frac{3}{4}$ to 4 inches in size. They can be had in almost any quantity merely for the asking and can be discarded when they become broken or soiled. This method should be regarded as only temporary.

Under this heading may be mentioned again the fact that in several herbaria where the vertical system of filing is used the boxes in which the larger specimens are to be placed are of the same lateral dimensions as the packets and are filed vertically in their proper sequence among them. The thickness of these boxes usually ranges from $\frac{1}{2}$ to $2\frac{1}{2}$ inches.

Where the smaller boxes are filed within the larger ones the genus is indicated on the end of the large box. The species labels are either placed loosely in the small boxes or are attached to them. The latter plan is usually followed if the specimens are subject to more or less constant use.

5. *Other Methods of Filing Specimens*

The methods already described are the ones principally used in the strictly mycological herbaria. However, in some of these and more especially in pathological herbaria some or all of the following additional methods are used. For purposes of demonstration certain specimens may be preserved in liquid, such as alcohol or formalin, in a glass container of appropriate size. Such specimens may be previously treated in such a manner as to preserve their natural colors. Other specimens suitable for demonstration may be enclosed in a Riker or similar mount; in special stationary cases of glass, or at least with glass doors; in shallow hinged cases which may be either open or fitted with

glass doors; still others in balsam, or some other suitable medium, on microscopic slides.

FILING CASES

It is not the intention to discuss in this paper the different types of filing cases that may be used in which to keep mycological specimens. However, it is thought that one or two illustrations may be of interest. Figure 4 shows a view of the herbarium cases and work tables in the Farlow Cryptogamic Herbarium at Harvard.[6] Plate 22 shows large metal cases on the main floor and in two galleries of the Gray Phanerogamic Herbarium at Harvard.[7] Although the Gray Herbarium is phanerogamic instead of cryptogamic the conservative use of space and the arrangement of the filing cases is of sufficient interest to make it worth while to include this illustration. These cases are arranged for the filing of the standard-size herbarium sheets in folders and would therefore be suitable for the filing of mycological specimens on similar sheets. At *A* and *B* on the gallery railings may be seen glass shelves upon which the folders are laid while specimens are being examined. Note also the desk or counter type of metal case, several of which are placed on the main floor. These cases as in the Farlow Herbarium are made as nearly as possible dust and insect proof; therefore they may be readily fumigated if such becomes necessary.

DISCUSSION

While some bulky mycological specimens are preserved in liquids they are usually either dried so that they will as nearly as possible retain their natural form, or they are cut or pressed so that they may be placed in a smaller container than if preserved whole. The filing of the whole specimen entails less work than the latter method, but it requires considerably more filing space. The objection is often raised that the cutting or pressing of the specimens destroys their natural size and form. However, if care is taken this can be very largely avoided if boxes no smaller

[6] The photograph for figure 4 was kindly furnished by Dr. Wm. H. Weston of the Laboratories of Cryptogamic Botany of Harvard University.

[7] The photograph for plate 22 was kindly furnished by Dr. H. M. Fitzpatrick of Cornell University, through the courtesy of the Art Metal Construction Company of Jamestown, N. Y., which made the cases.

than 4 × 6 inches and of appropriate thickness are used as containers for such specimens. By using a box of this size and by using discretion in cutting, a sufficient number of matched pieces can be preserved so that the form of the specimen may be retained. It is a rare case that the specimen is too large to be at least fairly satisfactorily preserved in this manner. Furthermore, the cutting of the specimen in this manner is considered by many to be an advantage rather than a disadvantage. For example, the cutting of a *Peridermium* specimen exposes the macroscopic effect of the parasite upon its host; the cutting of an *Agaricus* discloses the lateral surface of the gills and the gross structure of the pileus; and the cutting of a *Polyporus* permits of a lateral view of the pores.

Pressing a thick, bulky specimen is usually much more objectionable than cutting because of the resulting distortion. In the case of fungi on leaves or stems it would seem that there can be no real serious objection to the necessary reduction of the size of the larger specimens to the size of the standard container, provided the container is of such a size that it will meet the average requirements of minimum cutting and maximum conservation of filing space. It seems only reasonable that this should be an important governing factor in the filing of all specimens, whether flat or bulky, and whether filed flat on sheets or in boxes, vertically on half-sized sheets, or vertically in packets and boxes. Sooner or later every actively growing herbarium will demand a reduction in amount of filing space per individual specimen or the enlargement of the herbarium as a whole.

The neat and accurate folding of a sheet of paper into the form of a packet requires considerable skill and practice. The use of a pattern made of a thin but stiff paper card has been found to be very helpful. A piece of sheet aluminum of only sufficient thickness to give the required stiffness has also proved very satisfactory. The card or metal of the desired size is placed on the sheet of paper and the folds made accordingly. The pattern can be removed quickly by opening one end of the packet. This is especially true if a metal pattern is used; the weight of the metal will cause it to slide very easily and quickly from the

open end of the packet. These packets are later placed in a letter press or under a weight where the creases are made permanent. Many packets are made without the use of a pattern and thus probably a little more rapidly, but the time gained is

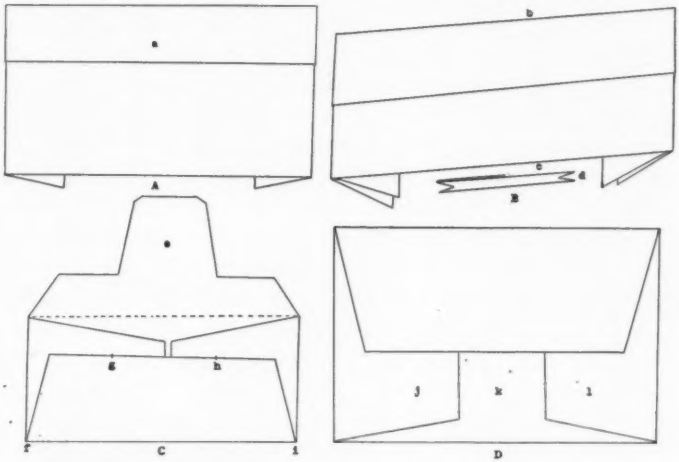

FIG. 3. Some of the different methods of folding packets which are used in mycological and pathological herbaria.

usually at the expense of neatness and accurateness. The flap which is folded over from the top and which is to bear the legend should preferably reach almost to the bottom of the packet. While this uses more paper it lends stiffness to the packet, thus protecting its contents, and also helps to prevent excessive bulging and gaping. Such a packet is also more easily put into the vertical file. If the packet is to be filed vertically the end flaps should be at least $1\frac{1}{4}$ inches long to facilitate filing and to prevent bulging, but if it is to be pasted or pinned to a herbarium sheet the ends should not exceed $\frac{3}{4}$ of an inch in length or there would be possible danger of injuring the specimen when opening or closing the packet.

Figure 3 illustrates the appearance and methods of folding four different types of packets. *A* is the type that is probably most commonly used in the United States. The length of the flaps at

the ends and the width of the marginal fold at *a* may be varied to suit individual needs. *B* is made on the bellows plan at the upper and lower edges. Such a packet is considerably more difficult to make, but is considered to be more flexible, to give more room for the specimen and also to give it more protection, being thicker at the edges. The drawing *d* represents a cross-section of *B* at *c b*. *C* represents a type of packet received from the Kew Garden herbarium. The flaps from *f* to *g* and *h* to *i* are sealed. The flap *e* is inserted under the portion *g* to *h* which is left unsealed. While there would be little danger of losing any portion of a specimen from such a packet it would be more difficult to insert or remove it. *D* is not an uncommon type of packet. None of the flaps are sealed and often are much shorter than indicated here. The flap *k* may be placed outside of *j* and *l* rather than as indicated in the drawing. Such packets have the advantage that they may be machine cut and folded, but there is more danger of the packet bulging open, especially if the end flaps are short.

In some herbaria it is a common practice to glue the specimen to the packet, to the box or to a card which is placed in a packet or box. While some specimens are so fragile that they require special care to prevent their injury it would seem that in at least most cases padding or pinning would be preferable to gluing. One of the values of a herbarium specimen rests in its accessibility for examination. In some cases sectioning of a specimen is desired and this is often difficult or impossible if it has been glued to its receptacle.

It is not the purpose of this paper to discuss the different classifications that may be followed in arranging mycological material. It may be stated, however, that as various herbaria differ in their methods of filing they also differ as to methods of classification arrangement. For example, in one herbarium the arrangement may be according to North American Flora, while in another it may follow Engler and Prantl. In at least one herbarium the specimens are filed and numbered according to Saccardo's Conspectus Systematicus Generalis, and Sylloge Fungorum. As the specimens are given their number a check is also made opposite the species in the publication and future references to a given specimen are made through this channel.

Perhaps other methods than those mentioned in this paper are used for filing mycological specimens, or perhaps there are modifications of these methods. If so, it is only further proof of

FIG. 4. A view of the filing cases and work tables in a portion of the Farlow Herbarium at Harvard University. (Photo by Dr. Wm. H. Weston).

the lack of uniformity which exists and which makes it difficult for visiting systematists or plant pathologists, to say nothing of those who constantly use them, to make efficient use of the herbaria. Probably the principal reasons for this lack of uniformity are (1) that in most institutions only a very limited amount of money is available for the building up of a herbarium, (2) often those who have started the herbarium have had little or no experience in such matters, and those who later take up the work are equally inexperienced or for financial reasons are forced to follow the original method, and (3) by the time the experienced curator is obtained the task necessary for the desirable rearrangement and revision is so great that the time required for their accomplishment is generally prohibitive.

At the present time it cannot be said that there is a "standard" method of filing and classifying mycological specimens. Possibly

such a condition will never exist because there is considerable room for difference of opinion as to just what the standard method should be.

The endeavor here has been to outline, without too much detail, the present methods and possibilities of filing specimens in mycological herbaria. The attempt has been made to present the matter in as nearly as possible an unprejudiced manner, the feeling being that the opinion of one man in such a matter would have comparatively little value. It is hoped, however, that those who are particularly concerned in such matters may be stimulated to further thought and discussion, in order that a greater uniformity in herbarium methods ultimately may be established. In the meantime it seems feasible to suggest that before rearrangement is begun in an already existing herbarium or in beginning a new herbarium, a careful study of the different methods in vogue at the present time should be made. Every herbarium curator should have in mind that it is his task to file the greatest number of specimens in a given space in such a manner that they will be most readily available for study, most nearly in natural form, and least likely to be damaged by handling or otherwise.

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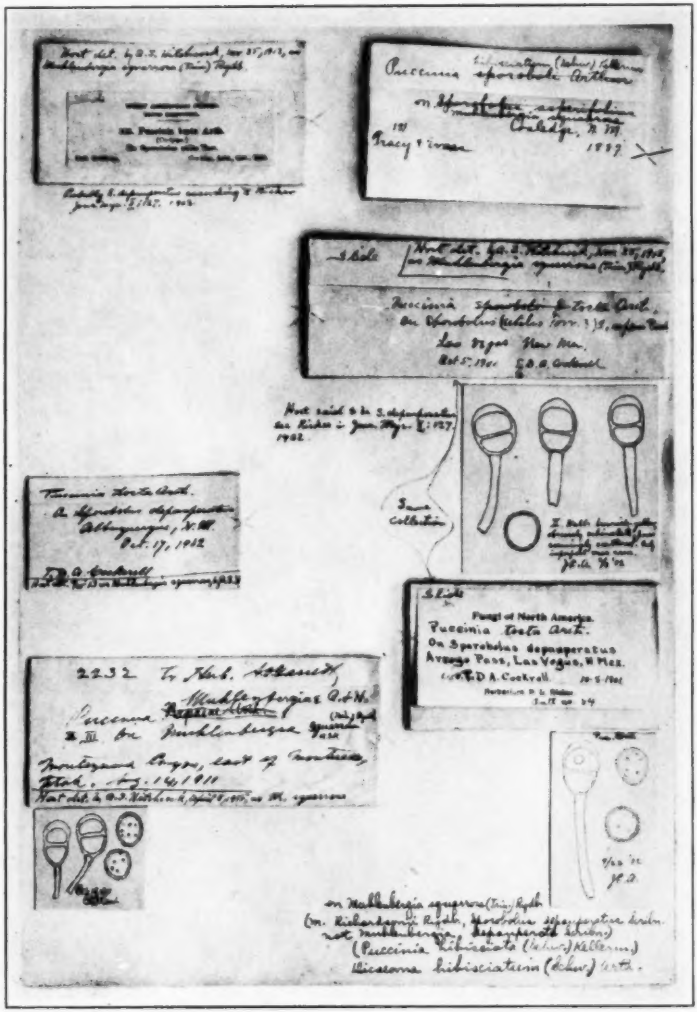
EXPLANATION OF PLATES

PLATE 21

Illustration of how packets, drawings, notes, etc., may be filed side by side on a single large herbarium sheet and thus be readily available for study at a moment's notice. (Photo by Dr. J. C. Arthur.)

PLATE 22

Type and arrangement of metal cases in the Gray Herbarium of Harvard University. Such cases are convenient and efficient for the filing of large herbarium sheets to which the small specimen packets are attached (Photo by Art Metal Construction Company).



SAMPLE SHEET FROM ARTHUR HERBARIUM

GRAY HERBARIUM, HARVARD UNIVERSITY

NOTES AND BRIEF ARTICLES

The explanation of Figures 9 and 10, Plate 24, Volume 22 of MYCOLOGIA should read as follows: 9. Ascus of *Physalospora Zeae* containing eight spores; 10. Ascus of *Physalospora Zeae* containing four perfectly formed spores.

Mr. Robert Hagelstein, Honorary Curator of Myxomycetes, in The New York Botanical Garden, sailed February 11th for a three weeks collecting trip in Trinidad and Barbados, his time having been chiefly devoted to Myxomycetes and Diatoms.

To correct a confusing error, the publishers, McGraw-Hill Book Company, Inc., 370 Seventh Avenue, New York, N. Y., have recently reprinted page 198 of my book, The Lower Fungi-Phycomycetes. A copy of this page will be mailed free by the publisher, or by the author, to every owner of the book who writes requesting it.—H. M. FITZPATRICK.

A Memoir of the Torrey Botanical Club (Volume 18, No. 1) recently issued consists of 108 page discussion by Professor Herbert S. Jackson of Toronto University on "Present Evolutionary Tendencies and the Origin of Life Cycles in the Uredinales." The paper was presented in part before the Mycological Section of the International Botanical Congress, held at Ithaca, New York, August, 1926.

The January issue of the American Journal of Botany contains an article by Ernst J. Schreiner on "Two species of Valsa causing disease in Populus." The two species reported as responsible for cankers in populars are *Valsa sordida* Nitschke, the pycnidial stage of which is *Cytospora chrysosperma* (Pers.) Fries and *Valsa nivea* (Hoff.) Fries with a conidial stage *Cytospora nivea* (Hoff.) Sacc. The disease caused by these two species of fungi are quite

similar. A great deal of experimental work has been conducted by Dr. Schreiner both in the greenhouse and in the field.

"Mycological Explorations of Colombia" by Carlos E. Chardon and Rafael A. Toro was issued in the Journal of the Department of Agriculture of Porto Rico **15**: 195–369. *pl. 30–35.* 1930. Several mycologists have collaborated in this work the Myxomycetes being treated by W. C. Muenscher; the Phycomycetes by W. H. Weston; the Pezizales by F. J. Seaver; the Xylariaceae by J. H. Miller; the Cercosporae by C. Chupp; the Ustilaginales by H. S. Jackson; the Uredinales by F. D. Kern and H. H. Whetzel and the Eubasidiomycetes by L. O. Overholts. A host index is appended. This is a very presentable contribution to our knowledge of South American fungi and includes the descriptions of many new species.